PHARMACOLOGICAL STUDIES OF OCTANAL RECOGNITION BY MAMMALIAN ODORANT RECEPTORS

by

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A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

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Abstract

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Research Advisor: Professor Kevin Ryan

The sense of smell is initiated when small molecule odorants bind and activate specific subsets of olfactory receptors (ORs) expressed by olfactory sensory neurons (OSNs). The mammalian nose is able to distinguish thousands of chemical structures through these receptors. The molecular recognition strategies that ORs use to bind and distinguish among different odorants are poorly understood. Here we use drug design techniques to probe olfactory molecular recognition using a model odorant-receptor pair *in vivo*. Specifically, octanal analogs were designed and synthesized to probe the steric and electronic requirements of octanal recognition by the aldehyde-specific rat OR-I7 in OSNs.

The testing of conformationally restricted octanal analogs (Chapter 1) indicates that OR-I7 distinguishes among aliphatic aldehydes according to their length and carbon chain conformation. A gauche conformation between C_4 and C_5 is proposed to be necessary to fill a small hydrophobic binding pocket 7 to 8 Å from the receptor-bound aldehyde with octanal's hydrophobic tail. In addition, small cycloalkyl groups at the tail enhance activation potency, leading to one analog that is more potent than octanal.

Several pairs of stereoisomers and analogs derived from cyclohexylacetaldehyde (Chapter 2) were also designed and synthesized to study the active conformation of octanal at C_4-C_5 and C_6-C_7 positions.

To study the possibility that OR-I7 and other aldehyde-specific olfactory receptors recognize, not the aldehyde, but its gem-diol hydrate, difluorooctanal, dimethyloctanal, octanol and octanal and were screened against more than 1,000 OSNs *in vivo*. 87 octanal-responsive OSNs were found. The response profiles of these cells support the hypothesis that for some ORs aldehydes function, by analogy to prodrugs, as pro-odorants, or "prodorants." The volatility of the aldehyde allows delivery to nasal mucous, where the aldehyde form is in equilibrium with the gem-diol, a form that has much richer H-bonding capacity.

Through these studies, some molecular recognition details of octanal by ORs were obtained, which could contribute to the understanding of general odorant-receptor recognition strategy.

To my parents

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I owe my deepest gratitude to my research mentor, Professor Kevin Ryan. Without his guidance, expertise, patience and kindness, this thesis would not have come to fruition. It has been an honor to work with him, and I will always be grateful for his help.

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Table of Contents

List of tables

List of Figures

Abbreviations

Å	Angstrom
Aq	Aqueous
d	Doublet
dd	Doublet of doublets
DIBAL-H	Diisobutylaluminum hydride
ee	Enantiomeric excess
eq or equiv	Equivalent
GC/MS	Gas chromatography-mass spectrometry
GPCR	G protein-coupled receptor
h	Hour
HRMS	High-resolution mass spectrometry
LAH	Lithium aluminum hydride
min	Minute
NMR	Nuclear magnetic resonance
OR	Odorant receptor
PCC	Pyridinium chlorochromate

Pd/C	Palladium on carbon
rt	Room temperature
s	Singlet
t	Triplet
THF	Tetrahydrofuran
TLC	Thin layer chromatography

Introduction

Sense of smell, together with sense of sight and hearing are the major senses that we use to detect the changes in the environment around us. Sight and hearing are our "physical" senses detecting light and sound of which the physical natures (wavelength for light, frequency for sound) are well understood. We gain information and develop media to spread information mostly through sight and hearing. However, until about 20 years ago, sense of smell was a mystery. As our "chemical" sense, smell or olfaction detect and distinguish odorants which are usually low molecular weight chemicals found in the surrounding environment. Unlike the other chemical sense taste which can only detect five basic taste: sweetness, bitterness, sourness, saltiness, and umami, our olfactory system can recognize thousands of different smells.

Sense of smell help us to sense the environment for food, danger or other individuals. Odors can be detected from a distance so that our brain gets an advanced warning about the environment. Smell plays sometimes more important role in animal world, especially those with poor sight. It helps animals to identify and communicate with each other.

The perception of odor starts when an odor molecule binds an odorant receptor in the nose from outside of the cell. The binding of odor molecule changes the olfactory receptor's conformation from inactive form to its active form. This triggers cascade events inside the cell that results in a signal being sent to the brain[1] (Scheme I.1). Humans have approximately 400 functional genes coding for olfactory receptors[2], but trained professionals (i.e. perfumers) can recognize and distinguish thousands of odors. To achieve this, olfactory system uses an olfactory code to distinguish different odorant. Each olfactory receptor can be activated by a number of structure related odorants[3], and each odorant can activate a number of olfactory receptors[4].

Moreover, an odorant can also bind to olfactory receptor but doesn't activate it, therefore competes with those that can activate the ORs. When we smell an odor which is caused by multiple odorants, the unique set of ORs that are activated and antagonized generate an olfactory code specific for the odor and processed by the brain.

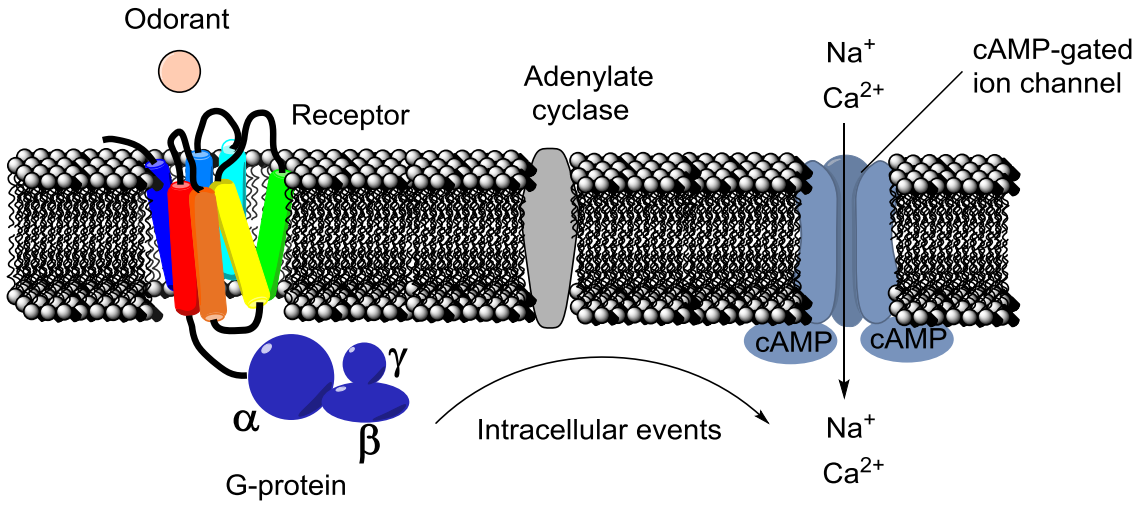


Figure I.1 Olfactory receptor signal transduction pathway

Technical difficulties have been obstructions on studying the detailed ligand-receptor recognition on molecular level. Olfactory receptors (ORs) belong to the large family of G protein-coupled receptors (GPCRs) which are also known as seven-transmembrane domain receptors. The membrane protein structure makes them hard to be separated and crystallized. There is still a lack of experimental structures at atomic level for olfactory receptors. To work around this problem, researchers have applied different approaches to obtain information about the ligand-receptor recognition including: 1. Computational simulation of ligand binding to the receptor based on the known structure of rhodopsin[5]; 2. Site-directed mutation of olfactory receptors and testing their activity[6]; 3. studying the electrical properties of the receptor[7]; 4. Chemistry approach to design, synthesis and test odorant analogues; 5. Combination of above methods[8].

A ligand-receptor pair has been previously deorphaned by Prof. Stuart Firestein's group at Columbia University[4c]. It was reported that: 1). OR-I7 specifically recognizes the aldehyde group. Replacing the aldehyde group with other functional groups including formate, hydroxyl, carboxylate, nitrile, thiol, amine and halide groups causes the molecule to lose the ability to activate OR-I7. 2). The length and the shape of the alkyl chain affect the activity of the compound. In this thesis, a medicinal chemistry approach is used here to understand how the weak intermolecular forces between receptor and ligand affect the binding and activation of olfactory receptors. Most odorants consist of a hydrocarbon chain and a hetero-atom-containing functional group with the hetero-atom usually being oxygen, sulfur or nitrogen. The hydrocarbon chain presumably interacts with olfactory receptors through van der Waals interactions and the hydrophobic effect, while the hetero-atom-containing functional group presumably interacts with olfactory receptors by polar interactions (i.e. hydrogen bonding and dipole-dipole attraction). The relative simple structure of octanal enables us to focus on these two portions of the molecule (Scheme I.2).

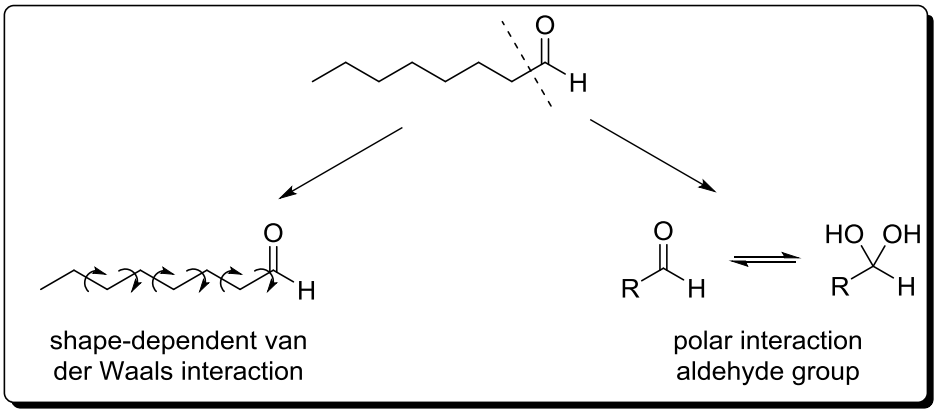


Figure I.2 The approach to study octanal recognition by odorant receptors

For the carbon chain, we focus on how octanal's different conformations are sensed by OR-I7. Conformational restriction strategy is used to lock the free rotation of carbon chain at

different range and different position. We aim to find a conformational frozen analog of octanal that represents octanal's active conformation when it binds to the receptor. If found, the relatively small loss of conformational entropy upon binding should result in increased binding potency, i.e., the ligand is said to be "pre-organized". For the carbonyl group, we focus on how its hydration affects octanal's activity. We test the hypothesis that aldehydes diffuse through the air in the more volatile aldehyde form, but in the nose, in some cases, the olfactory receptor may recognize the hydrated form as it has greater hydrogen bonding ability.

Understanding the recognition of OR-I7 and octanal can be meaningful. It not only helps to understand how olfactory system distinguishes different odors, but also increases understanding of how GPCRs recognize their ligands in general. 30-50% of FDA approved drugs target GPCRs[9], so the understanding of molecular recognition is very helpful in drug design. Octanal itself and many structure related aldehydes have floral and fruity smell, and are used as fragrances in perfumes and in flavor production for the food industry. The study of the structure activity relationship of octanal can be useful in studying and designing aldehyde fragrances.

Chapter 1: The importance of octanal conformation to the binding and activation of OR-I7

1.1 Introduction

This Chapter was published in *Chemistry & biology*, *15* (12), Peterlin, Z.; Li, Y.; Sun, G.; Shah, R.; Firestein, S.; Ryan, K., The importance of odorant conformation to the binding and activation of a representative olfactory receptor, 1317-27.

To understand the olfactory code at the chemical level will require a precise understanding of the chemical determinants responsible for activating and blocking each OR. Several studies have cited molecular ‘‘length’’ as one such determinant[1]. Length studies have focused mainly on odorants containing aliphatic carbon chains[1a, 1c-e]. These studies used homologous series of conformationally flexible *n*-alkyl acids, aldehydes, ketones, and alcohols. However, the conformational flexibility of such odorants leaves unclear the true molecular length required for activation because aliphatic odorants exist in large ensembles of conformational isomers. This uncertainty also raises the question whether ORs bind odorants in preferred conformations—such as an extended conformation, as implied in the previous studies—but disfavor the same odorants when presented in other conformations. Moreover, GPCR binding and GPCR activation may have different conformational requirements. To address the variable of odorant conformation as a factor in the molecular receptive range of a representative OR, we have assayed a series of conformationally restricted analogs of octanal, the primary agonist for the rat I7 olfactory receptor (OR-I7). Testing these compounds has provided insight into the activation and blocking of the OR-I7 receptor, and has demonstrated how conformational flexibility influences the total number of ORs activated by a single odorant.

1.2 Results

The rat OR-I7 receptor is one of the few ORs to have been cloned, expressed in neurons, and functionally characterized by probing with a large collection of odorants[1c, 2]. OR-I7 is activated by multiple aliphatic aldehydes having a length between ~8 Å and ~12 Å[1c]. We note that multiple conformations are possible for aliphatic aldehydes. We therefore define length here to mean the length of the longest attainable (and typically lowest energy) conformation (see Experimental Procedures). The most potent OR-I7 ligand found thus far is octanal, referred to hereafter as **C8** (for 8 carbon *n*-alkanal; likewise for **C7**, **C6**, etc.). Like many ORs, OR-I7 is activated by odorants with successive carbon chain lengths centered on the most potent ligand[1a, 1d].

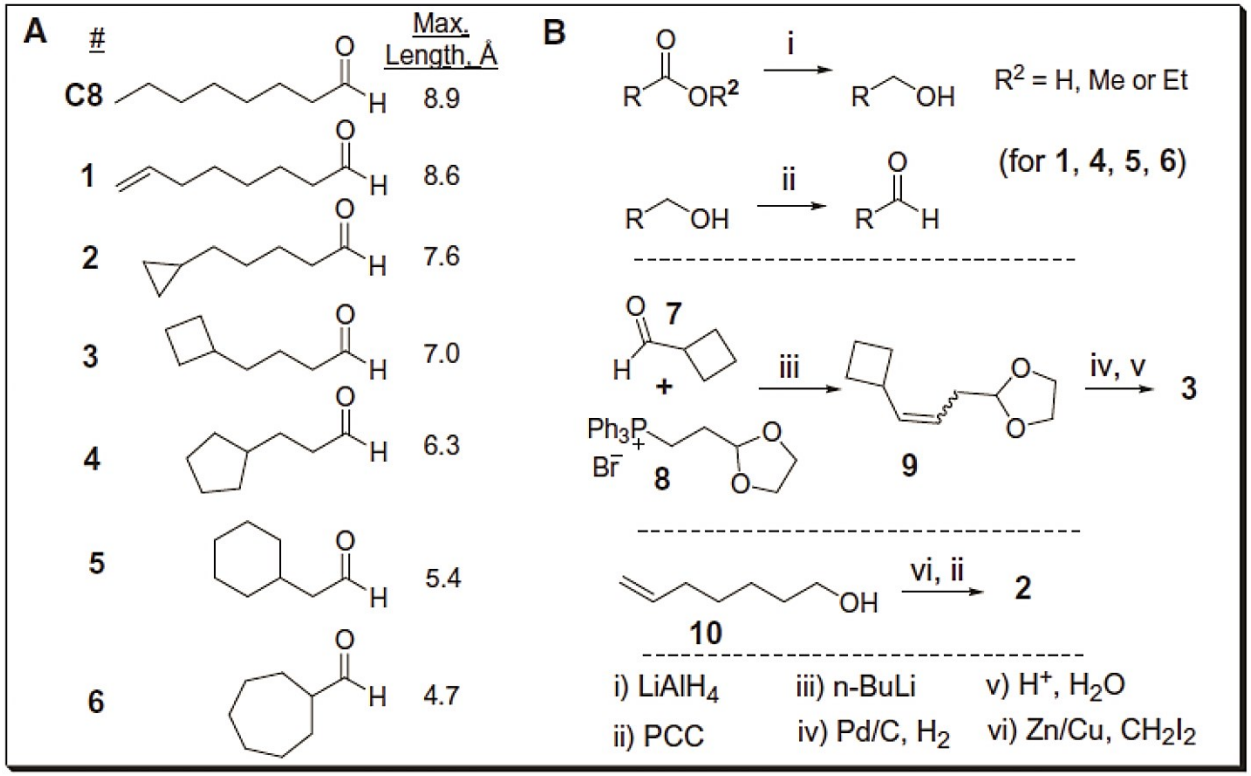


Figure 1.1 Conformationally restricted octanal analogs (A) Lengths refer to the distance measured from the carbonyl carbon to the most distant carbon as described in experimental procedures. (B) Synthetic routes to compounds **1-6**.

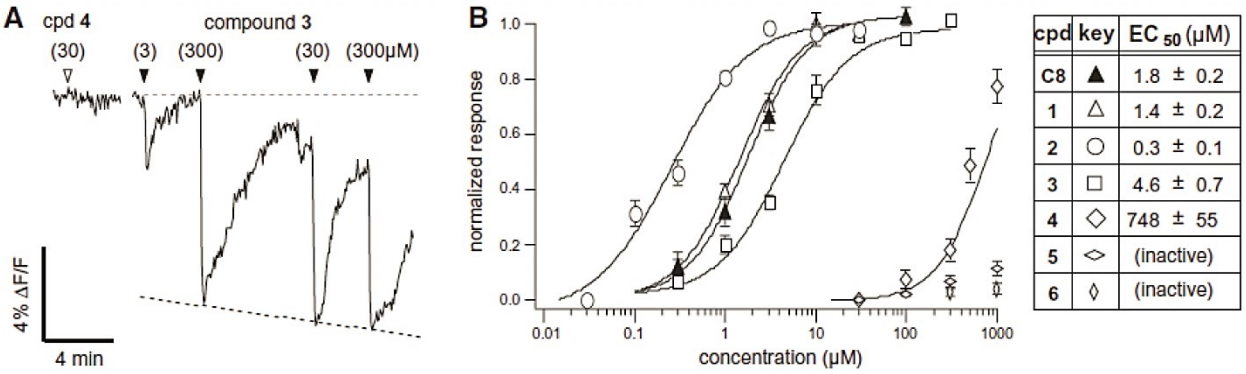
In the rat nasal epithelium, **C8** activates more cells and elicits a greater cAMP (the signal transduction second messenger) response than do shorter and longer homologs[1d], indicating that the dimensions of the OR-I7 binding site are likely close to average. OR-I7 is thus typical and well characterized, ideal for a systematic investigation of the effect of odorant conformation on its receptive range.

A Series of Conformationally Restricted Eight-Carbon Aldehydes

C8 is highly flexible, having six rotatable bonds that can each adopt three different conformations: one anti, or one of two gauche. The maximum number of formally possible conformational isomers is $3^6 = 729$, though symmetry makes some equivalent and undoubtedly reduces this number. Nothing is known about the bound conformation of **C8**. On the one hand, were **C8** to bind and activate OR-I7 in one or a small subset of favored conformers, it would incur a conformational entropy penalty in the free energy of binding due to the loss of conformational flexibility. In this case, preorganizing **C8** to resemble the bound conformation should improve binding by minimizing the loss of entropy. On the other hand, a previous study compared the calculated lowest energy conformation of a group of activating ligands and concluded that OR-I7 may tolerate a number of structural variations at the carbons most distant from the aldehyde[1c], possibly indicating that many different **C8** conformers are capable of activating OR-I7. To gain insight into the activating conformation(s) of **C8**, we made a series of eight-carbon aldehydes with restricted conformations (Figure 1.1A). Conceptually, carbon 8 (denoted as C_8) of **C8** was tied back by establishing a new bond successively to C_7 through C_2, yielding compounds **1**–**6**, respectively. Unlike the previously studied series of homologous *n*-alkanals, in which the partition coefficient and other physical properties can vary with the number of carbons in the chain, we expect to maintain throughout our eight-carbon series similar

physical-chemical properties while reducing the number of possible conformations. This strategy should enable us to study effects that occur at the level of the OR binding pocket while minimizing receptor independent effects. In this series the maximum length of the aldehydes is also progressively shortened. Due to the conformational restriction, the maximum length is now a better estimation of this dimension compared with the *n*-alkanal series. The synthesis of these analogs was straightforward and is summarized in Figure 1.1B.

OR-I7 Activation: Octanal Uses a Semi-Extended Conformation



cpd	key	EC 50 (μM)
C8	▲	1.8 ± 0.2
1	△	1.4 ± 0.2
2	○	0.3 ± 0.1
3	□	4.6 ± 0.7
4	◇	748 ± 55
5	<>	(inactive)
6	◊	(inactive)

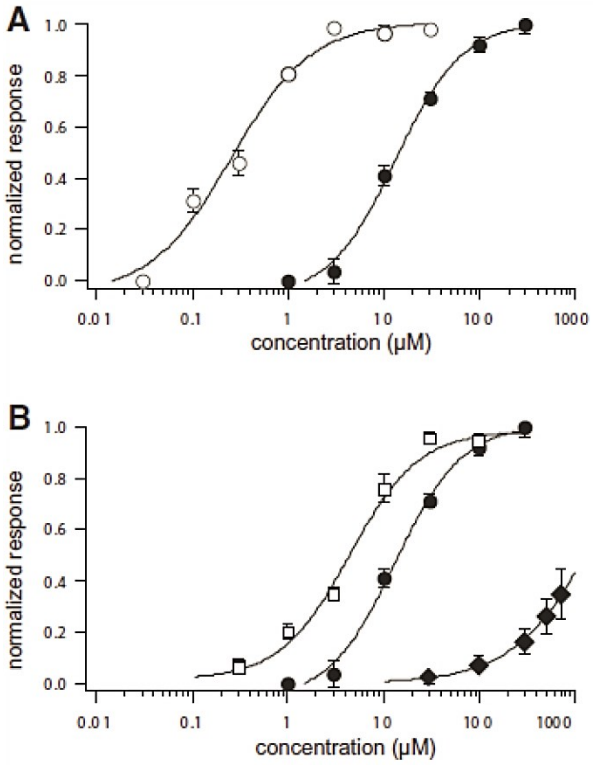
Figure 1.2 OR-I7 activation by cyclic octanal analogs (A) Calcium imaging traces from a GFP+ OSN, showing how OR-I7 responds at a near saturating level to 30 μM of compound **3** but is unresponsive to compound **4** at the same concentration. Grey dashed line denotes baseline; black dashed line denotes a trend lien for normalization. (B) Activation dose-response curves for the cyclic compound series (open symbols). The activation dose-response curve for octanal (**C8**) is also provided for reference (filled symbol). Octanal and compounds **1**-**3** are saturated over this range and thus normalized to their respective maximal responses. Compounds **4**-**6** are shown normalized to the response to 10 μM octanal. The maximal efficacies for each compound, relative to 10 μM **1**, were as follows (mean ± SEM): **2**, 0.99 ±0.002; **3**, 1.06 ± 0.1; **C8**, 0.89 ± 0.002; and **C7**, 0.86 ± 0.07. Data point error bars represent ±SEM; EC_{50} ± SD (biological testing courtesy of Zita Peterlin and Stuart Firestein, Columbia University)

The eight-carbon aldehydes were tested via calcium imaging of dissociated rat neurons expressing recombinant OR-I7 from an adenoviral vector as previously described[2c]. The cells were loaded with fura-2AM (a fluorescent label for Ca^{2+} finding) followed by application of the testing compounds. When a cell is activated by a compound, Ca^{2+} channels on the cell membrane will open. The increased Ca^{2+} that form complex with Fura-2 (the removal of acetoxymethyl

esters of Fura-2AM inside the cell generates Fura-2) induce a change of fluorescence which is recorded by image software[3] As an example, the activation of OR-I7 by analog **3** is shown in Figure 1.2A. Responses were concentration dependent and saturating. At high concentrations, the magnitude of the response to analogs **1**, **2**, and **3** saturated with efficacies (maximum effect) comparable to that of **C8**; no partial agonists (maximum effect less than octanal's maximum effect) were detected. Analogs **4**, **5**, and **6** failed to reach saturation over this concentration range. Activation curves for the entire series, including **C8**, are shown in Figure 1.2B, which also tabulates the concentrations at which half-maximal activation is reached (EC_{50}). The compounds segregate into two groups. Compounds **1**, **2**, and **3**, which have smaller rings and four to six freely rotatable bonds, all strongly activated OR-I7, whereas compounds **4**, **5**, and **6**, which contain larger rings and one to three rotatable bonds, activated OR-I7 weakly or not at all. The greatest difference in activity, 163-fold, was observed between compound **4** (6.3 Å, EC_{50} = 748 mM) and compound **3** (7.0 Å, EC_{50} = 4.6 mM). The *n*-alkanals of 5–12 carbons (**C5**–**C12**) were previously tested against OR-I7 in the vapor phase using electroolfactogram (EOG) recordings[1c, 2b]. By that method, the largest difference in activity in the series fell similarly between **C6**, (no activation, 6.4 Å) and **C7** (activation, 7.6 Å). Thus, using the new series of **C8** analogs, we confirmed that there is a minimum length requirement for activation, and further narrow it down from 6.4–7.6 Å to 6.5–6.9 Å. We interpret the finding that an aldehyde of only 7.0 Å is sufficient to activate the receptor, compared with the extended length of **C8** (8.9 Å), to mean that **C8** does not activate OR-I7 in its fully extended conformation, but rather adopts one or more semi-extended conformations to do so. The poor activity in the eight-carbon aldehydes **4**–**6**, where the variables of total carbon number and length are separated, defines the shorter end of the

activating length cutoff, and provides evidence that **C8** does not activate OR-I7 while in compact conformations approximating those mimicked by **4**–**6**.

Small Cycloalkyl Rings Enhance OR-I7 Activation



C

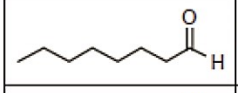
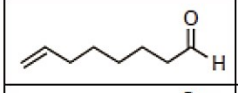
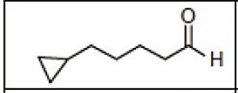
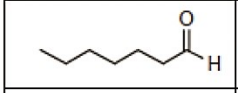
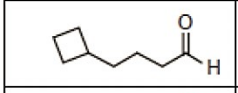
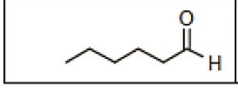
structure	cpd	key	max length (Å)	EC_{50} (μM)
	C8	▲	8.9	1.8 ± 0.2
	1	△	8.6	1.4 ± 0.2
	2	○	7.6	0.3 ± 0.1
	C7	●	7.6	12 ± 2.5
	3	□	7.0	4.6 ± 0.7
	C6	◆	6.4	1736 ± 437

Figure 1.3 Cyclopropyl and cyclobutyl ring-containing analogs are more potent than predicted from their maximal lengths (A) Activation dose-response curves for cyclic compound **2** (open circles) and the *n*-aldehyde of identical length, **C7** (filled circles). Data point error bars represent ± SEM. (B) Activation dose-response curves for cyclic compound **3** (open squares) and the *n*-aldehydes of flanking lengths **C7** (filled circles) and **C6** (filled diamonds). Data point error bars represent ± SEM. (C) Summary of maximal lengths and EC_{50} of activation for the strongly activating cyclic and *n*-aldehydes. The relative activation of **C8** and compound **1** can be found in Figure 1.2B

To test whether maximum length is solely responsible for the difference in activity observed among compounds **1**–**6**, we obtained the full activation curves for **C7** and **C6** by calcium imaging (Figure 1.3). Although **C7** and compound **2** have identical extended lengths, **2** was 40-fold more potent (Figure 1.3A). Compound **2** was even more potent than **C8**.

Similar to previous OR-I7 EOG recordings[1c], calcium imaging revealed a sharp increase in activity (145-fold) in the step from **C6** to **C7** (Figure 1.3B). The maximum length of compound **3**, which contains the cyclobutyl group, falls between those of **C7** and **C6** (Figure 1.3C). Based on a correlation with maximum length, the activity of **3** should also fall between that of **C7** and **C6**. However, **3** was more potent than both (Figure 1.3B), providing a second example where a small cycloalkyl ring increased potency beyond what was expected based on length alone. Thus, though the activity of the cyclic compounds generally required a certain minimum length, restricting the rotation of the terminal two or three bonds enhanced potency, indicating that specific conformations or shapes at the end opposite the aldehyde are preferred by the activating form of OR-I7.

An Activating Octanal Conformation

We next explored conformational restriction of **C8** toward the middle of the chain. In examining the data shown in Figure 1.2B, we noted that all of the active compounds had a rotatable bond C_4-C_5, whereas in all inactive compounds this bond was locked in a ring. Although this observation might merely reflect the variable of length, in another study the same bond in *trans*-2-*cis*--6-nonadienal, an OR-I7 activating compound, was implicated as a potential pivot point important for activation[1c]. In the extended conformation, all of **C8**'s C-C bonds adopt the anti conformation (Figure 1.4A, left). Rotation of the C_4-C_5 bond by 120° into a gauche conformation (Figure 1.4A, middle) reduces the total length of **C8** from 8.9 Å to 8.0 Å, provided the other bonds remain in the anti conformation. Changes of this nature could serve to reduce the actual length of the molecule into the type of semi-extended conformation proposed herein. To test the effect of this particular alteration, we installed a two-carbon bridge from C_3 of **C8** to C_6 (Figure 1.4A, right). The resulting six-member ring locked **C8** into a gauche conformation

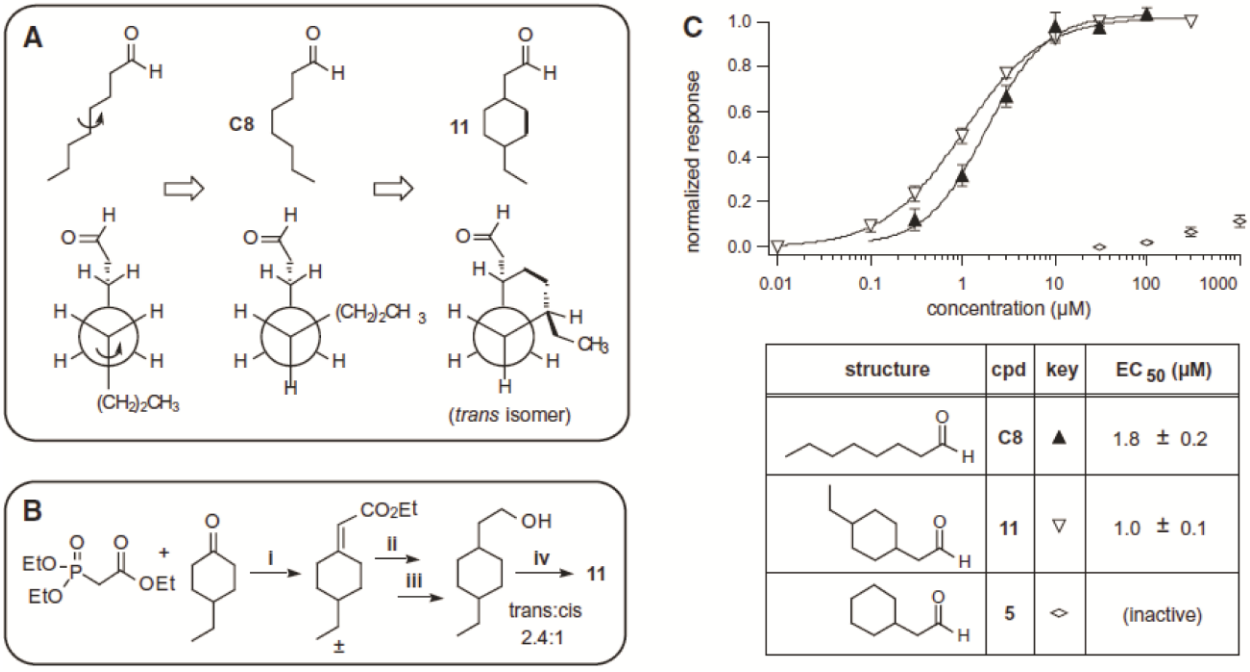


Figure 1.4 A semiextended octanal conformation activated OR-I7 (A) Line structures (top) and Newman projections (bottom) depicting rotation around the C_4-C_5 bond in octanal, and how it was locked in the gauche conformation in compound **11**. Only the *trans* isomer's New man projection is shown. (B) Synthetic route to compound **11**. (C) Activation of dose-response curves for octanal (**C8**, filled triangles) and *cis*/*trans* mixture of compound **11** (open inverse triangles). The related compound **5** which lacks the 4-ethyl group, has no substantial activity (open compressed diamonds). The maximal efficacy for **11** was 1.07 ± 0.05, relative to 10 μM octanal. Data point error bars represent ±SEM; EC_{50} ± SD

around C_4-C_5. Imagining this process beginning with a rotation of the same bond in the opposite sense produces the same structure, due to symmetry. Compared with the 729 hypothetical conformations that **C8** can sample, the resulting **C8** analog, **11**, can exist in only ~10 closely related conformers. **11** was synthesized as a ~2.4:1 mix of the *trans*:*cis* isomers, as outlined in Figure 1.4B and described in Experimental Procedures.

Unable to separate these two isomers, we tested **11** as a mixture. In the case where one isomer is inactive—or perhaps even an antagonist—testing the mixture incurs the risk of underestimating the true response of the other isomer. Despite this concern and the introduction of a six-member ring into the middle of **C8**, the isomeric mixture of **11** was more active than **C8**, shifting the activation curve slightly to the left (Figure 1.4C). Just as **C8** is two carbons longer

than the much less potent **C6**, compound **11** is two carbons longer than the nearly inactive 5, and the gain in activity might appear to correlate merely with the increase in extended conformation length (the *cis* isomer of **11** is ~7.4 Å and the *trans* is ~8.0 Å). However, in contrast to **C8**, the two terminal carbons of **11** are fixed by the ring in their relation to the aldehyde group, though in slightly different locations in the two isomers. If we assume that the orientation of the aldehyde group with OR-I7 is fixed in the odorant binding site, then the three-dimensional coordinates of the ethyl group of **11** must likewise be fixed and occupy a distal (to the aldehyde) activating region in the receptor. **11** may therefore resemble an, or the, activating conformation of C8, just as **4**, **5**, and **6** are constrained to resemble inactive conformations.

Conformational Determinants of OR-I7 Antagonism

In nature, odorants are typically encountered in mixtures. In this context, each odorant can activate one set of receptors while simultaneously antagonizing a subset of receptors activated by other components, leading to great complexity in the olfactory code at the level of sensory input[1a, 1c, 2c, 4]. Most OR antagonists discovered to date are structurally related to the agonists whose activity they suppress[1c, 2c, 4]. Interestingly, natural product fragrances typically contain structurally related odorants[5], suggesting a potential evolutionary significance.

We thus set out to systematically probe the length and conformation requirements for antagonism of OR-I7 by simultaneously applying a saturating concentration of **C8** (10 mM) and increasing concentrations of either the inactive **C8** analogs **5** and **6** or the similarly inactive **C4** and **C5**. The marginally active **C6** and **4** were also used. Unexpectedly, nearly all were capable of antagonizing **C8** activation, suggesting a broad antagonist receptive field with regard to the hydrophobic portion of short aldehydes. A representative calcium imaging trace is shown in

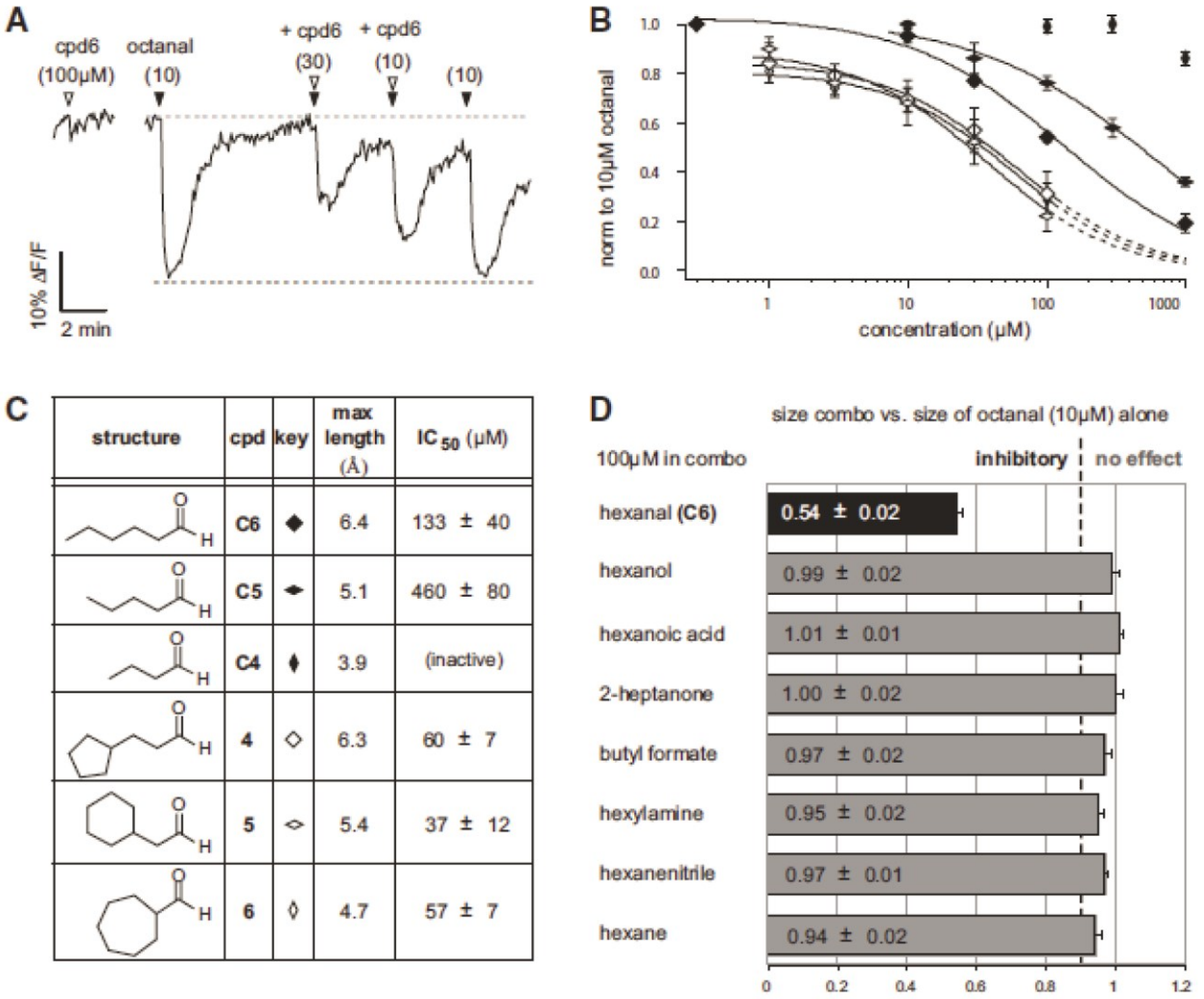


Figure 1.5 Inhibition of OR-I7 Activation by Short Octanal Analogs (A) Calcium imaging traces from a GFP+ OSN showing the dose-dependent antagonism of compound **6** against a saturating dose of octanal. Black arrowheads denote the application of 10 μM octanal either with or without coapplication of **6** (open arrowheads). The black dashed line is the trend line, indicating the predicted response magnitude if the coapplication had no effect. (B) Inhibition dose-response curves for cyclic analogs and *n*-aldehyde of similar lengths, tested at various concentrations against a 10 μM octanal stimulus. The cyclic compounds (open symbols) all display very similar potencies regardless of length, whereas the *n*-aldehyde (filled symbols) show length dependence for antagonism. Dashed lines indicate extrapolation used to estimate IC_{50}. Data point error bars represent ± SEM. (C) Summary of maximal lengths and IC_{50} values for the antagonizing aldehydes. IC_{50} ± SD. (D) An aldehyde groups is required for OR-I7 antagonism. Nonaldehydes of similar size were unable to antagonize octanal activation of OR-I7. Dashed line indicates 90% of the signal produced by 10 μM octanal alone. Error bars represent ± SEM.

Figure 1.5A. Here, analog **6**, which itself cannot activate OR-I7, is shown to antagonize **C8** activity. Inhibition curves for **4**, **5**, **6**, **C4**, **C5**, and **C6** are shown in Figure 1.5B with the

concentration of each required for 50% inhibition (IC_{50}) tabulated in Figure 1.5C. Among the *n*-aldehydes, antagonist potency increased with the number of carbons in the chain. The failure of **C4** (3.9 Å) to antagonize **C8** activation may indicate a minimum *n*-aldehyde chain length requirement for antagonism between 4.0 Å and 5.1 Å, but we cannot rule out receptor-independent effects, such as reduced hydrophobicity and increased water solubility due to the small size. Among the cycloalkyl ring-containing aldehydes, where the constant number of carbons should control for receptor independent effects, all were moderate antagonists but without apparent length dependence (Figure 1.5C). In fact, the IC_{50}s for the cyclic compounds were remarkably similar and each was a more potent antagonist than its closest length-matched *n*-alkanal. This result may indicate a dependence of antagonism on odorant surface area or carbon number in combination with a maximum length below 6.5–6.9 Å. Taken together with the activation data, aldehydes that resemble **C8** in a compact conformation appear to be able to bind OR-I7 in its unactivated state, blocking subsequent activation by **C8**, whereas those that can extend beyond 6.5–6.9 Å appear able to bind and stabilize OR-I7 in its activated state. Held close to the aldehyde, the large cycloalkyl groups appeared to enhance antagonism, just as the small cycloalkyl rings, held distant, enhanced activation. Overall, these results provide an example where a structural trait, namely maximum attainable length, is correlated in a systematic way with the transition from antagonism to agonism.

1.3 Conclusion

The molecular recognition of airborne chemicals is challenging because volatility requires low molecular weights and a minimum or absence of polar functional groups, yet this is the subset of chemical space that the olfactory receptors (ORs) have been charged by evolution to monitor. Nearly all odorants have rotatable bonds and can adopt multiple conformations. In a

representative system, we have investigated the variable of octanal conformation as a molecular determinant of OR-I7 activation and antagonism. We show that OR-I7 binds a variety of aliphatic aldehydes, but then applies length and conformational criteria that lead either to activation (longer than 6.5–6.9 Å) or antagonism (shorter than 6.5–6.9 Å). Using a series of octanal mimics, we chart the transition from antagonism to agonism as a function of increasing length. For octanal, the apparent primary agonist for this receptor, we deduce that long and short

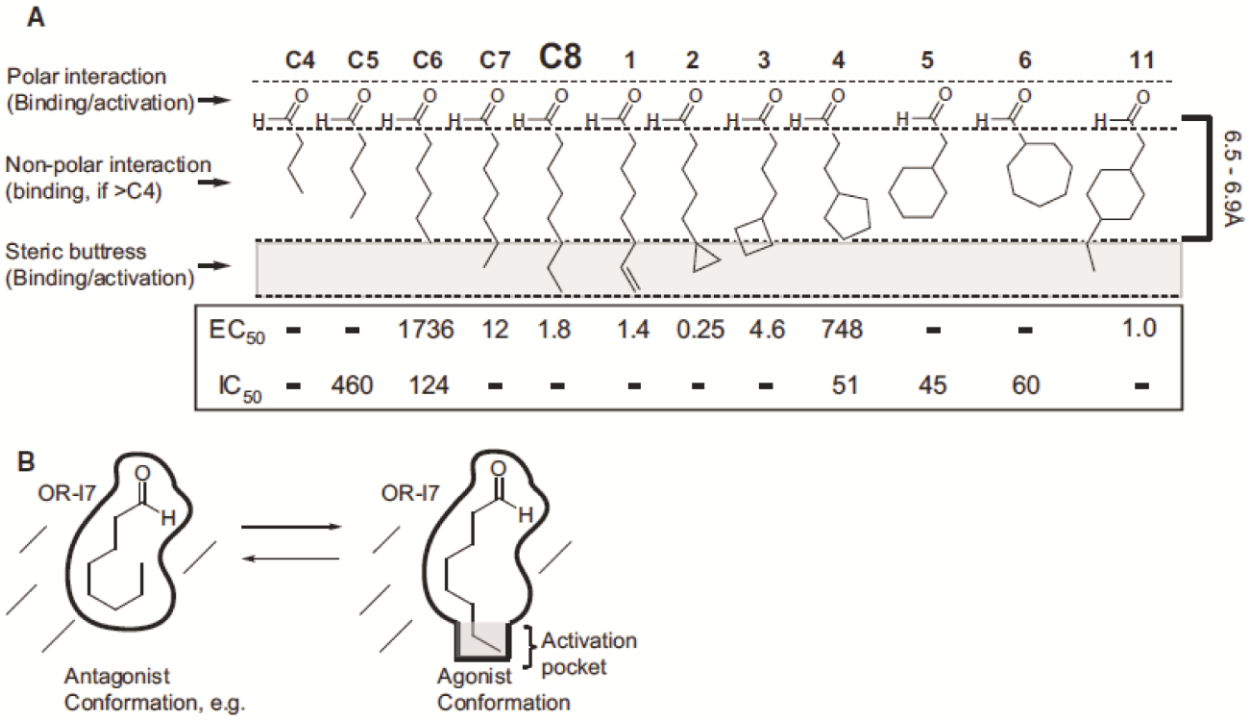


Figure 1.6 Summary of OR-I7 Binding and Activation by Octanal Conformation Mimics (A) Structures, maximum lengths, and inhibition/activation constants. Regions of the structures responsible for binding and activation are indicated (left), as is the 6.5-6.9 Å length requirement for activation (right). Except for C4, which had neither type of activity, dashes in the IC_{50} row indicate that the compound was not tested for antagonism because it is strongly activating. Dashes in EC_{50} row indicate the compound had no activity within its solubility range. (B) Schematic depiction of octanal's conformation on OR-I7's activation.

conformers bind the resting state of OR-I7 and, through a double-induced fit, cooperate to produce the activating odorant-OR pair. In mixtures, various OR-I7-bound aldehydes, whether activating or antagonizing, contribute to the olfactory code either positively or negatively,

enabling I7 to respond in a gradual manner to mixtures of aliphatic aldehydes rather than to only the best-tuned ligands. For OR-I7, we also find that small cycloalkyl groups at the distal end of an aldehyde enhance activation potency. We propose that they fit into and buttress a small hydrophobic pocket present only in the activated form of the receptor, sterically preventing reversion to the unactivated form. The steric buttress may be a common strategy for recognizing nonpolar odorants, such as the hydrocarbons.

1.4 Experimental Procedures

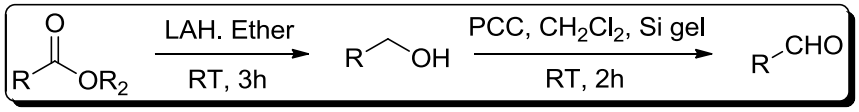
Method to estimate the maximum extended length of aldehydes: Chem3D ultra 10.0 was used. The structure of the aldehyde was drawn in its most extended conformation. The energy was minimized using MM2 forcefield. The length was then measured from the carbonyl carbon to the furthest carbon from it.

Materials and General Methods: Commercial reagents and solvents were used without additional purification, unless otherwise noted. 3-cyclopentylpropanoic acid, cyclobutanemethanol, ethyl 2-cyclohexylacetate, 2-(2-bromoethyl)-1,3-dioxolane, 4-ethylcyclohexanone were purchased from Alfa Aesar. Cycloheptanecarboxylic acid was purchased from Frinton Laboratories, Inc. 7-octen-1-ol and 6-hepten-1-ol were purchased from TCI America. Ether and THF were dried and distilled from Na/benzophenone. Analytical thin layer chromatography (TLC) was performed on precoated silica gel 60 plates. Column Chromatographic purifications were performed using 230-400 mesh silica gel from Alfa Aesar.

Compound Characterization

^{1}H NMR spectra were obtained at 300 MHz. Chemical shifts are reported in parts per million (ppm) referenced to the appropriate solvent peak. The following abbreviations were used to describe peak patterns when appropriate: br = broad, s = singlet, d = doublet, t =triplet, q = quartet, m = multiplet, dd = doublet of doublet, dt = doublet of triplet.

Coupling constants, *J*, are reported in Hertz (Hz). ^{13}C NMR was recorded at 75 MHz. Infrared (IR) spectra were recorded using Thermo Nicolet 6700 FT-IR Spectrometer, and are reported in wavenumbers (cm^{-1}). GC/MS analyses were performed on a Shimadzu GC/MS QP5000 with GC-17A Gas Chromatograph (capillary column: DB-1-30N-STD). The temperature program for GC analysis: program1: held at 50 °C for 1min, heated from 50 to 150 °C at 3 °C/min,, and held at 150 °C for 4min; program 2: held at 60 °C for 1min, heated from 60 to 150 °C at 4 °C/min, and held at 150 °C for 4min. Mass spectra for compounds **9**, **2a**, **11a**, **14** and **15** were recorded on JOEL LCmate mass spectrometer at Columbia University Chemistry Department Mass Spectrometry Facility.



R	R^2	RCH_2OH(**a**)	RCHO
6-heptenyl	N/A	N/A	**1**
4-cyclopropylbutyl	N/A	**2a**	**2**
2-cyclopentylethyl	H	**4a**	**4**
Cyclohexylmethyl	Et	**5a**	**5**
Cycloheptyl	H	**6a**	**6**
(4-ethylcyclohexyl)methyl	Et	**11a**	**11**

Figure 1.7 LAH reduction and PCC oxidation to aldehyde

General Procedure for Lithium Aluminum Hydride (LAH) Reduction [6]**.** To an ice-cold mixture of LAH (1.5 equiv for acids, 1.2 equiv for esters, 0.038g/ mL) in dry ether under N_2, was added dropwise acid or ester (1 equiv, 0.2g/ mL) in dry ether. The mixture was stirred at room temperature for 3 h then re-cooled to 0° C. The mixture was worked up by dropwise and sequential addition of X ml H_2O, X ml 15% NaOH solution and 3X ml H_2O (X= grams of LAH used). After stirring for few minutes, the mixture was filtered through a celite pad and washed with ether. The solution was dried and concentrated under reduced pressure. The crude can be purified by column chromatography with ether/hexane (3:7), but was typically used without further purification.

3-Cyclopentylpropanol (4a). Now available from Aldrich**.** Prepared from 3-cyclopentylpropanoic acid. Yield: 80%. IR (thin film, NaBr plates), ν: 3331, 2949, 2867, 1452, 1057 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.60 (t, *J* = 6.7, 2H), 1.86–1.63 (m, 4H), 1.63–1.39 (m, 6H), 1.39–1.18 (m, 2H), 1.15–0.90 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 63.4, 40.1, 32.9, 32.3, 32.2, 25.3; GC retention time: 15.16 min (program 1)

2-Cyclohexylethanol (5a) [7]**.** Prepared from ethyl 2-cyclohexylacetate. Yield: 85%. IR (thin film, NaBr plates), ν: 3331, 2923, 2852, 1448, 1051 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.65 (t, *J* = 6.5, 2H), 1.78–1.54 (m, 5H), 1.51–1.31 (m, 4H), 1.27–1.05 (m, 3H), 0.99–0.79 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 61.0, 40.5, 34.4, 33.5, 26.7, 26.4; GC retention time: 15.04 min (program 1)

Cycloheptylmethanol (6a). Now available from Aldrich**.** Prepared from cycloheptanecarboxylic acid. Yield: 84%. IR (thin film, NaBr plates), ν: 3332, 2922, 2854, 1460, 1075, 1020 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.39 (d, *J* = 6.5, 2H), 1.81–1.28 (m, 12H), 1.15

(dd, J = 11.2, 21.0, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 68.8, 42.2, 30.9, 28.7, 26.7; GC retention time: 12.13 min (program 2)

General Procedure for Pyridinium Chlorochromate (PCC) Oxidation [8]**.** To a stirred suspension of PCC (1.5 equiv,) and silica gel (1 g/ g of PCC) in CH_2Cl_2 (10 mL/ g of PCC) was added a solution of the alcohol (1 equiv, 0.083 g/ mL) in CH_2Cl_2. The mixture was stirred at room temperature for 2 h and then filtered through a silica gel pad which was rinsed thoroughly with fresh solvent. The solvent was evaporated and the crude product was purified by column chromatography with ether/hexane (1:19) to give the aldehyde product.

7-Octenal (1) [9]**.** Prepared from 7-octen-1-ol. Yield: 61%. IR (thin film, NaBr plates), ν: 3077, 2932, 2858, 2719, 1728, 1641, 996, 911 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.76 (s, 1H), 5.78 (dt, J = 6.9, 16.2, 1H), 4.81-5.09 (m, 2H), 2.42 (t, J = 7.3, 2H), 2.17–1.86 (m, 2H), 1.74–1.50 (m, 2H), 1.10-1.49 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.9, 138.8, 114.7, 44.0, 33.7, 28.7, 22.1; GC retention time: 12.75 min (program 2)

5-Cyclopropylpentanal (2). Prepared from **2a.** GC/MS shows that the product contains 4% heptanal, likely from heptanol as a by-product from the Simmons-Smith reaction. It could not be removed by column chromatography. Yield: 50%. IR (thin film, NaBr plates), ν: 3077, 3001, 2928, 2856, 2718, 1728, 1481, 1015, 822 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.73 (s, 1H), 2.39 (t, J = 7.3, 2H), 1.74–1.51 (m, 2H), 1.48–1.31 (m, 2H), 1.17 (dd, J = 7.1, 14.5, 2H), 0.70–0.50 (m, 1H), 0.35 (q, J = 4.8, 2H), -0.05 (q, J = 4.8, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.1, 44.1, 34.6, 29.4, 22.1, 10.8, 4.5; GC retention time: product: 11.60 min, (Contaminating heptanal: 7.19 min (program1)).

3-Cyclopentylpropanal (4). Prepared from **4a**. Yield: 50%. IR (thin film, NaBr plates), ν: 2950, 2867, 2717, 1727, 1452 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.76 (t, *J* = 1.7, 1H), 2.43 (td, *J* = 1.7, 7.8, 2H), 1.85–1.37 (m, 9H), 1.08 (br, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.2, 43.5, 39.8, 32.7, 28.3, 25.3; GC retention time: 12.03 min (program 1); LRMS (EI): 126(M^+).

2-Cyclohexylacetaldehyde (5) [10]**.** Prepared from **5a**. Yield: 57%. IR (thin film, NaBr plates), ν: 2934, 2852, 2713, 1728, 1449, 1020, 899 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.74 (s, 1H), 2.27 (d, *J* = 6.7, 2H), 1.98–1.54 (m, 6H), 1.39–0.81 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.2, 51.6, 33.4, 32.8, 26.2; GC retention time: 13.99 min (program 2)

Cycloheptanecarbaldehyde (6) [11]**.** Prepared from **6a.** Yield: 50% IR (thin film, NaBr plates), ν: 2926, 2856, 2707, 1728, 1460, 1182, 888 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.63 (s, 1H), 2.44–2.30 (br, 1H), 2.02–1.85 (m, 2H), 1.81–1.35 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.9, 52.0, 28.7, 27.4, 26.4; GC retention time: product: 13.22 min. (The product contained ~4% unknown contaminant by NMR and GC/MS). Retention time: 13.22 min (program 1)

2-(1,3-dioxolan-2-yl)ethyl)triphenylphosphonium bromide (12) was prepared by refluxing 2-(2-bromoethyl)-1,3-dioxolane (5 g, 27.6 mmol) and triphenylphosphine (7.24 g, 27.6 mmol) in 25 mL acetonitrile for 24 h. The mixture was then cooled to room temperature. Dry ether was added and the starting material in the ethereal solution was removed by decantation. This procedure was repeated 3 times and the resulting phosphonium salt was dried under vacuum to give 11g 2-(1,3-dioxolan-2-yl)ethyl)triphenylphosphonium bromide. Used without further purification.

2-(3-Cyclobutylallyl)-1,3-dioxolane (3a) [12] 1.6 M *n*-BuLi solution in hexane (Alpha Aesar) (11.6 mL, 18.5 mmol) was added dropwise to a stirred suspension of **12** (8.2 g, 18.5 mmol) in

dry THF (200 mL) at –75 °C under N_2. The orange solution was stirred for 1 h then **13** (see next entry) (1.41 g, ~16.8 mmol) in dry THF (150 mL) was added dropwise. The mixture was then allowed to warm to room temperature and continued to stir overnight (12 h). The reaction was quenched by adding saturated NaCl solution and the triphenylphosphine oxide was removed by filtration. The aqueous phase was extracted with ether and combined organic phase was washed with brine and dried ($MgSO_4$). Evaporation of the solvent and purification by column chromatography using ether/hexane (1:19) furnished the compound (1.5 g, 53%). IR (thin film, NaBr plates), ν: 2960, 2880, 1397, 1136, 1036, 944, 842; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.65 (ddt, *J* = 1.6, 8.7, 10.3, 1H), 5.27 (dtd, *J* = 1.3, 7.3, 10.8, 1H), 4.82 (t, *J* = 4.8, 1H), 4.11–3.60 (m, 4H), 3.30-3.02 (m, 1H), 2.37 (ddd, *J* = 3.9, 6.3, 11.1, 2H), 2.21-1.99 (m, 2H), 1.95–1.54 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.8, 121.0, 104.2, 65.1, 34.0, 32.6, 29.8, 19.1; GC retention times: 16.36 min (major isomer) and 16.42 (minor isomer) (program 2); *Cis*/*trans* ratio 8:1; LRMS (APCI+): 169 (M^++1).

Cyclobutanecarbaldehyde (13) Prepared by PCC oxidation of cyclobutylmethanol. Most methylene chloride solvent was removed from the filtered crude product by simple distillation followed by adding excess ether and removing the ether also by simple distillation. Repeated ether treatment one time. Only a trace of methylene chloride (by ^{1}H-NMR) was left after this treatment. Used without further purification.

2-(3-Cyclobutylpropyl)-1, 3-dioxolane (3b). A suspension of **9** (1.5 g, 8.93 mmol) and palladium on carbon (Acros, 10% Pd on carbon, 50% wet with water, 0.15 g) in ethyl acetate (60 mL) was stirred under H_2 for 2 h at room temperature. The mixture was then filtered. Evaporation of the solvent furnished the product (1.4 g, 92%) which was used in the next step without further purification. IR (thin film, NaBr plates), ν: 2948, 2863, 1410, 1142, 1050, 944;

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.81 (t, *J* = 4.8, 1H), 4.04–3.75 (m, 4H), 2.34–2.10 (m, 1H), 2.09-1.91 (m, 2H), 1.90–1.67 (m, 2H), 1.66–1.46 (m, 4H), 1.44–1.19 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 104.9, 65.0, 37.1, 36.2, 34.1, 28.5, 21.9, 18.6; GC retention time: 16.74 min (program 2); LRMS (APCI+): 171 (M^++1), 169 (M^+-1).

4-Cyclobutylbutanal (3). A mixture of (1 g, 5.88 mmol) in 60mL 2 N HCl and 60 mL THF was refluxed with stirring for 3 h. The mixture was cooled to room temperature and extracted with ether. The combined organic layers were extracted with saturated $NaHCO_3$ solution, brine and dried ($MgSO_4$). Evaporation of the solvent and purification of the crude by column chromatography using CH_2Cl_2/pentane (1:4) furnished the aldehyde (0.25 g, 34%). IR (thin film, NaBr plates), ν: 2933, 2860, 2717, 1727; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.75 (s, 1H), 2.39 (t, *J* = 7.2, 2H), 2.32 -2.16 (m, 1H), 2.12–1.94 (m, 2H), 1.91-1.71 (m, 2H), 1.68–1.25 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.1, 44.0, 36.5, 35.9, 28.4, 19.9, 18.6; GC retention time: 11.72 min (program 1).

5-Cyclopropylpentanol (2a) [13] 6-hepten-1-ol (2 g, 17.5 mmol), diiodomethane (18.76 g, 70.0 mmol) in 8 mL dry ether were added to a mixture of Zinc-Copper couple (made according to reference [24] (6.84 g) and 0.44 g I_2 in 8 mL dry ether at room temperature. After 20 min, an exothermic reaction occurs and the mixture started to reflux. After 15 min the exothermic reaction subsided, the reaction was gently heated to keep refluxing for another 30 min. The mixture was then transferred to a sealed heavy walled glass tube (CAUTION: It's crucial to wait for the exothermic reaction to occur before transferring the mixture to the sealed tube, and keep the temperature below 70 °C. Otherwise the reaction may cause explosion!), flushed with N_2 and sealed. The reaction was stirred at 55 °C for 18 h. NMR showed that 70% of the olefin was converted to the cyclopropyl group. The mixture was diluted with $CHCl_3$, filtered through celite

pad and washed with saturated NH_4Cl solution. The aqueous solution was extracted with $CHCl_3$. The combined organic layers were dried and concentrated under vacuum to give 3.6 g crude product which contained ether and acetal byproducts [14].

The unreacted 6-hepten-1-ol could not be separated by column chromatography but was evident by NMR. We therefore used hydroboration to convert it into a more polar compound that could be separated: To a solution of the crude product (3.6 g in 15 mL dry THF) was added 1 M BH_3 in THF (2.98 mL, 2.89 mmol) dropwise. After 10 min, 50% Na_2CO_3 solution (1.74 mL) was added, followed by 30% H_2O_2 (1.31 mL). The mixture was stirred for 2 h. 5 mL H_2O was added and the layers were separated. The aqueous layer was washed with ether. The combined organic layers were washed with brine and dried ($MgSO_4$). The solvent was evaporated, and the residue (3 g) was used directly in the next step. ^{1}H-NMR showed that all the olefin had been consumed.

To convert the acetal side products into the alcohol prior to oxidation, the crude product was refluxed in 100 mL 2 N HCl and 100 mL THF for 4 h. The mixture was cooled and extracted with ether. The combined organic layers were washed with saturated $NaHCO_3$ solution, saturated NaCl solution and dried ($MgSO_4$). Evaporation of the solvent and purification of the crude by column chromatography using CH_2Cl_2 furnished the alcohol 2a (1 g, 45%). GC/MS shows that the product contains 4 % (inseparable) heptanol of unknown origin. IR (thin film, NaBr plates), ν: 3332, 3076, 3000, 2928, 2855, 1462, 1056, 1014; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.59 (t, J = 6.6, 2H), 1.86 (s, 1H), 1.53 (dt, J = 6.7, 13.7, 2H), 1.45–1.23 (m, 4H), 1.16 (dd, J = 6.9, 13.8, 2H), 0.51-0.70 (m, 1H), 0.35 (ddd, J = 3.9, 5.6, 8.0, 2H), 0.02–-0.16 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 63.1, 34.9, 33.0, 29.6, 25.8, 11.0, 4.5; GC retention time: product: 14.72 min, heptanol impurity: 9.75 min (program 1); LRMS (APCI+): 129 (M^++1).

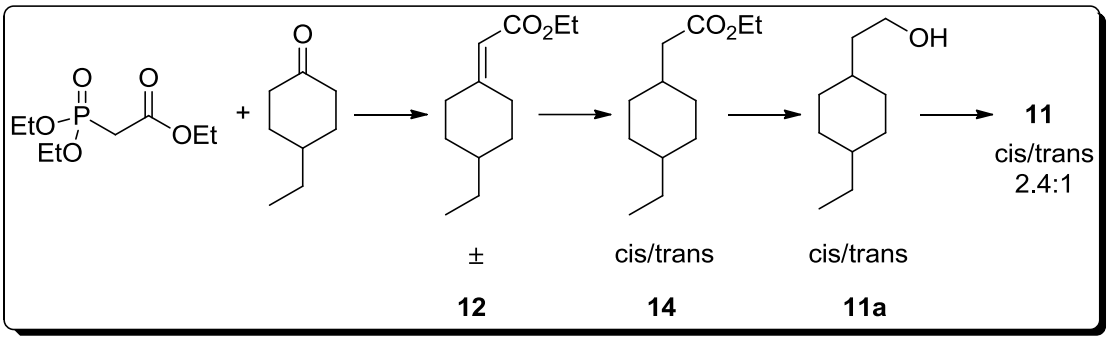


Figure 1.8 Preparation 2-(4-Ethylcyclohexyl)acetaldehyde

Ethyl 2-(4-ethylcyclohexylidene)acetate (12). Ethyl 2-(diethoxyphosphoryl)acetate (8.88 g, 39.6 mmol) in 15 mL dry THF was added dropwise to NaH in 50 mL THF. After the addition, the reaction mixture was stirred for 1 h at room temperature. 4-ethylcyclohexanone (5 g, 39.6 mmol) in 10 mL dry THF was added at a rate that the temperature of the mixture was kept below 30 ° C. The solution was then stirred for 15 min during which time a viscous semi-solid appeared. The mixture was taken up in a large excess of water and the aqueous solution was extracted with ether. The ether layer was dried and concentrated. The crude product was purified by column chromatography using ether/hexane (1:19) to give racemic **12** (6.84 g, 88%). IR (thin film, NaBr plates), ν: 2933, 2855, 1716, 1650, 1186, 1150; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.58 (s, 1H), 4.12 (q, *J* = 7.1, 2H), 3.73 (d, *J* = 14.2, 1H), 2.34–2.03 (m, 2H), 2.01–1.79 (m, 3H), 1.46–1.31 (m, 1H), 1.30–1.14 (m, 5H), 1.12–0.95 (m, 2H), 0.87 (t, *J* = 7.4, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.0, 163.8, 113.1, 59.6, 39.1, 37.5, 34.3, 33.6, 29.1, 14.5, 11.8; GC retention time: 29.22 min (program 1)[15].

Ethyl (4-ethylcyclohexyl)acetate (14). Racemic **12** (5.3 g, 27 mmol) was hydrogenated with the procedure described above to give ethyl 2-(4-ethylcyclohexyl)acetate (5.1 g, 95%, mixture of *cis* and *trans* isomers). IR (thin film, NaBr plates), ν: 2961, 2920, 2852, 1736, 1174;

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.10 (q, *J* = 7.1, 2H), 2.33–1.93 (m, 2H), 1.82–0.60 (m, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.6, 173.4, 60.2, 42.4, 39.3, 37.2, 35.3, 33.1, 32.6, 30.0, 28.9, 28.4, 14.4, 12.0, 11.6; (The ^{13}C NMR of compounds **14**, **11a** and **11** show more than the expected number of resonances due to the presence of *cis* and *trans* isomers. The ratio of the two isomers was determined to be ~2.4:1 by GC/MS (See entry for compound **11** for assignment of major and minor isomers). GC retention time: 26.78 min and 27.15 min in 2.4:1 ratio (program 1). LRMS (APCI+): 199 (M+H)+.

2-(4-Ethylcyclohexyl)ethanol (11a). Prepared by LAH reduction of compound 14 (see General procedure above). The product is a mixture of *cis* and *trans* isomers. The ^{1}H NMR of the product shows two partial overlapping triplets at δ 3.78-3.57 in about 2:1 ratio (-CH2OH). Yield: 93%. IR (thin film, NaBr plates), ν: 3331, 2960, 2920, 2852, 1448, 1047; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.78–3.57 (m, 2H), 1.85–0.67 (m, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 61.4, 61.0, 40.5, 39.6, 34.6, 33.4, 32.9, 31.9, 30.2, 29.1, 28.6, 12.0, 11.7; GC retention times: 22.08 and 22.63 min (program 1, broad trailing peaks); LRMS (EI): 156 (M+).

2-(4-Ethylcyclohexyl)acetaldehyde (11). Prepared from **11a** by the General PCC oxidation procedure described above. The product is a mixture of *cis* and *trans* isomers in 2.4:1 ratio based on GC/MS. The closest literature precedent[16] reported that the catalytic hydrogenation of ethyl 2-(4-tert-pentylcyclohexylidene acetate yielded 70.6% *trans* isomer and 23.4% *cis* isomer. We tentatively assign the *trans* isomer to be the major product. Proof that this is the correct *cis* and *trans* assignment will be presented in Chapter 2. IR (thin film, NaBr plates), ν: 2980, 2920, 2852, 2712, 1727, 1448; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.73 (s, 1H), 2.45–2.02 (m, 2H), 1.92–0.60 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.3, 51.5, 39.2, 33.3, 33.1, 32.7, 30.0, 29.1, 28.3, 11.6;

GC retention time: 18.74 min (*trans*) and 19.28 min (*cis*) in 2.4:1 ratio (program 1); LRMS (EI): 154(M+).

Chapter 2: Active conformation of octanal recognized by OR-I7

2.1 Introduction

OR-I7 is known to be activated by octanal and some aldehydes that are structurally related to octanal. To study the importance of octanal conformation to the binding and activation of OR-I7, conformationally restricted octanal mimics were designed and tested. The octanal mimics that resemble the active conformation of octanal are expected to be more potent than octanal because they lose less entropy upon binding and activation. Using this strategy, it was found that even though different conformations of octanal seem to bind to OR-I7, only a subset of them can activate OR-I7. The length of the aldehyde works as a determinant to distinguish agonists from antagonists, while certain conformation (e.g. small cyclic rings) of the aldehyde modifies the activity[1].

A potential problem with the compounds tested in Chapter 1 and previous tests is that most of those compounds are still quite flexible. They can bind to OR-I7 in their non-fully-extended forms, as the slightly higher energy can be easily overcome by the conformational energy of the receptor-ligand complex. How the conformation at different positions along the carbon chain contributes to OR binding and activation remains unclear.

4-ethylcyclohexylacetaldehyde (compound **2.1** in Figure **2.1**) with C_4-C_5 bond locked in gauche conformation was tested in Chapter 1 and found to resemble an activating conformation of octanal. The initial testing by Firestein et al.[2] suggested that the carbon chain around the carbonyl head up to C_4 is sterically constrained. If we assume that the carbon chain from C_1-C_5 is fixed, then only C_5-C_6 bond and C_6-C_7 bond can rotate to give <10 different conformations. The tail of the carbon chain, controlled by the conformation of these two bonds, is supposed to

interact with the receptor via hydrophobic and Van der Waals interactions and stabilize the activated form of the receptor. In this chapter, the conformations of C_5-C_6 and C_6-C_7 bonds are locked to explore the location and the size of the "activation pocket".

2.1.1 Design of Octanal analogues to study C_5-C_6 bond conformation

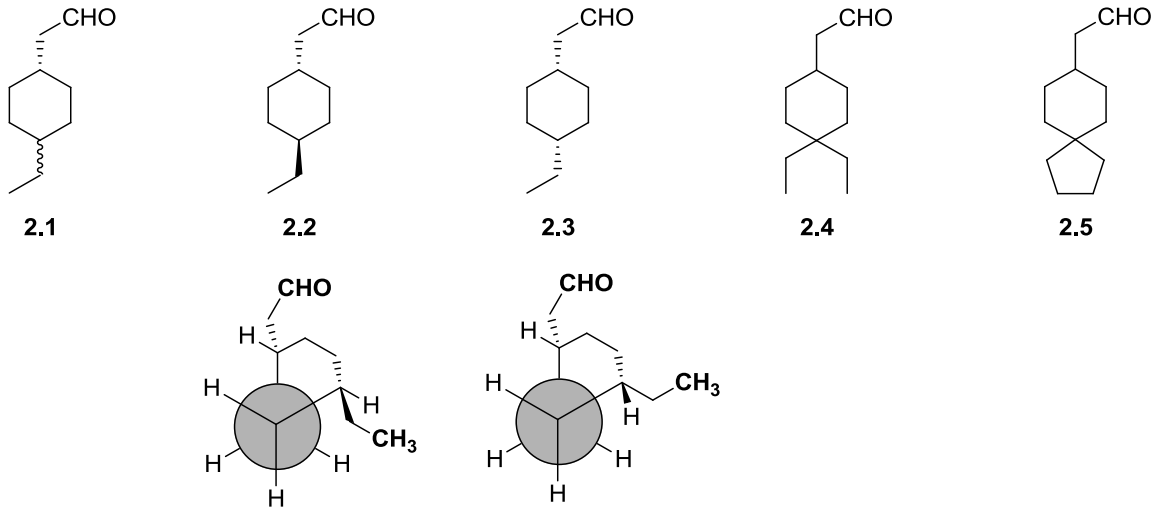


Figure 2.1 Conformation analogues of octanal to study C_5-C_6 bond conformation

Compound **2.1** was synthesized and tested as a mixture of *cis* and *trans* isomers (see Chapter 1). Its *cis* and *trans* isomers (compound **2.2** and **2.3**) have dihedral angle of 60° and 180° respectively at C_5-C_6 bond; and can be considered two analogues with C_5-C_6 bond rotation being locked. As a result, the end ethyl group in *cis* and *trans* isomers reaches into different receptor locations relative to the aldehyde group (Figure **2.1**). Since the ethyl group was found to be critical for activating OR-I7[1], it's quite possible that the *cis* and *trans* isomers in the mixture show different activities: one being a very potent agonist and the other being less potent or even act as an antagonist. Therefore, testing the two individual isomers and to find the more active one would be very helpful to locate the activation pocket. Unable to separate the two isomers from the mixture, they were synthesized separately.

It's also possible that both *cis* and *trans* isomers can equally activate OR-I7. In that case, would there be only one activation pocket that is big enough to fit both ethyl groups at the same time? Or, would there be two activation pockets? Would the activation of both ethyl groups additive? To answer these questions, the compound **2.4** and **2.5** that have both ethyl groups (free rotating or locked) were designed.

2.1.2 Design of octanal analogues to study C_6-C_7 bond conformation

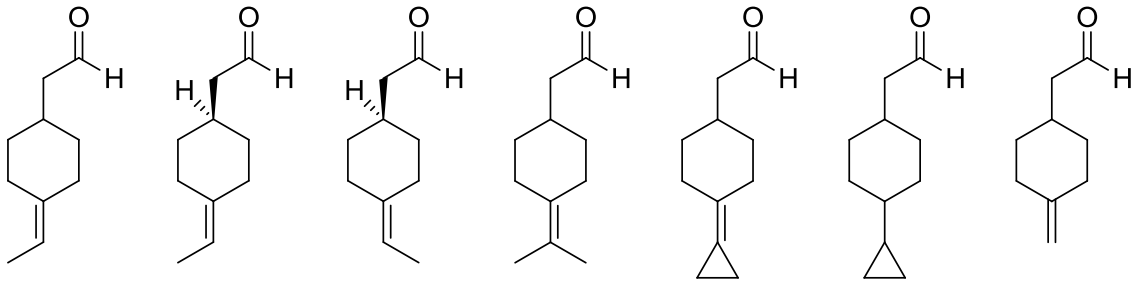


Figure 2.2 Conformation analogues of octanal to study C_6-C_7 bond conformation

In compound **2.2** and **2.3** above, the last free rotating C_6-C_7 bond points the C_8 methyl group to 3 different areas. To lock this bond without adding extra bulkiness, a C=C bond is introduced between C_6 and C_7. Assuming that the chain from C_1 to C_4 is fixed in the receptor, the conformation of the whole molecule becomes frozen. The resulting chiral compound 2-(4-ethylidenecyclohexyl)acetaldehyde (compound **2.6**) has two enantiomers: **2.7** and **2.8** with slightly differently positioned C_8 methyl group. It should be noted that the C=C bond changes the position of C_7. C_7 is at equatorial position in compound **2.2** and at axial position in compound **2.3**. In this group, the C_7 sits in between those two positions. Compound **2.12** lacking C_8 is then used as a control compound for this group to test whether the C_8 methyl group is required to activate the receptor and how much it can affect the potency. Similarly to the spiro compounds

2.4 and **2.5** in the previous group, compound **2.9** includes both C_8 methyl groups in the same structure.

One goal of this chapter is to find the ideal conformation of octanal that activates OR-I7. The corresponding mimic that represents this conformation will best activate OR-I7. We have attempted to combine the two structures that were found to enhance the potency of octanal: cyclopropyl ring and the structure of compound **2.1**. Therefore, compound **2.11** (*cis*/*trans* mixture) and compound **2.10** (C_6-C_7 locked) were made.

2.2 Synthesis of Conformational Analogues of Octanal

2.2.1 Synthesis of *cis*-(4-ethylcyclohexyl)acetaldehyde (compound 2.3)

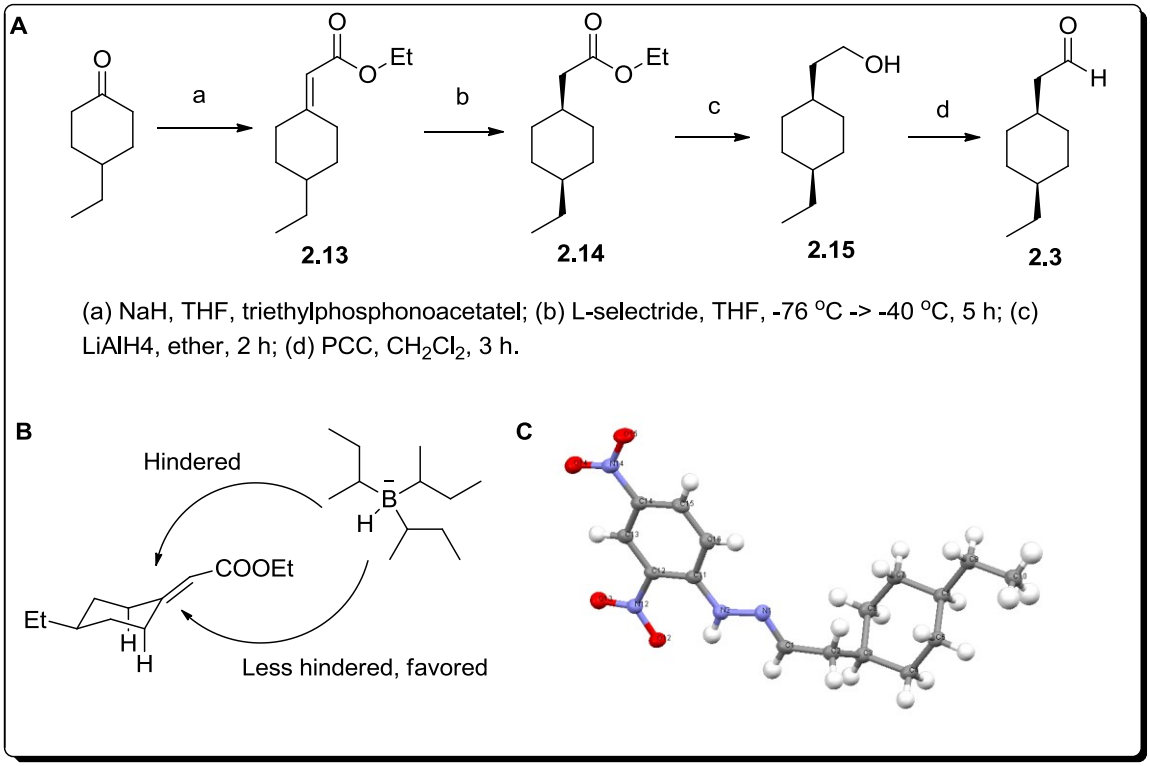


Figure 2.3 A: synthesis of *cis*-(4-ethylcyclohexyl)acetaldehyde; B: mechanism of conjugate reduction by L-selectride; C: Crystal structure of DNP derivative of *cis*-(4-ethylcyclohexyl)acetaldehyde

The synthesis of compound **2.3** is shown in Figure **2.3A**. α,β-unsaturated ester **2.13** was prepared by a Horner-Wadsworth-Emmons reaction of 4-ethylcyclohexanone. The key step to make the 1,4-*cis* structure was done by a stereo-selective reduction of ester **2.13** by L-selectride[3]. The stereo-selectivity of this conjugated reduction was accomplished by the attack of the bulky borohydride from the less hindered equatorial side (Figure **2.3B**). The resulting *cis* ester **2.14** was converted to the *cis* aldehyde **2.3** by LAH reduction and PCC oxidation.

A crystalline sample of compound **2.3** derivative was prepared by reacting **2.3** with 2,4-dinitrophenylhydrazine(DNP) and recrystallization. The crystal structure of the DNP derivative (Figure **2.3C**) confirmed the 1,4-*cis* configuration of aldehyde **2.3**.

2.2.2 Synthesis of *trans*-(4-ethylcyclohexyl)acetaldehyde (compound 2.2)

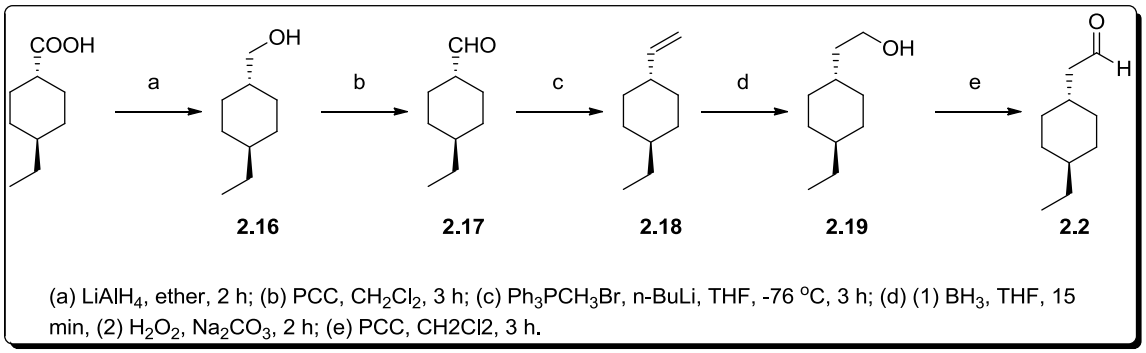


Figure 2.4 Synthesis of *trans*-(4-ethylcyclohexyl)acetaldehyde

Fortunately, there is a commercial available compound that already has the 1,4-*trans* substituted cyclohexane structure: *trans*-(4-ethylcyclohexyl)acetic acid. The acid was converted to aldehyde **2.17** by LAH reduction and PCC oxidation. Wittig reaction extended the aldehyde **2.17** by one carbon to afford alkene **2.18**. The alkene **2.18** was converted to terminal alcohol **2.19** by hydroboration and oxidation. The *trans*-(4-ethylcyclohexyl)acetaldehyde **2.2** was then prepared from the alcohol **2.18** by PCC oxidation.

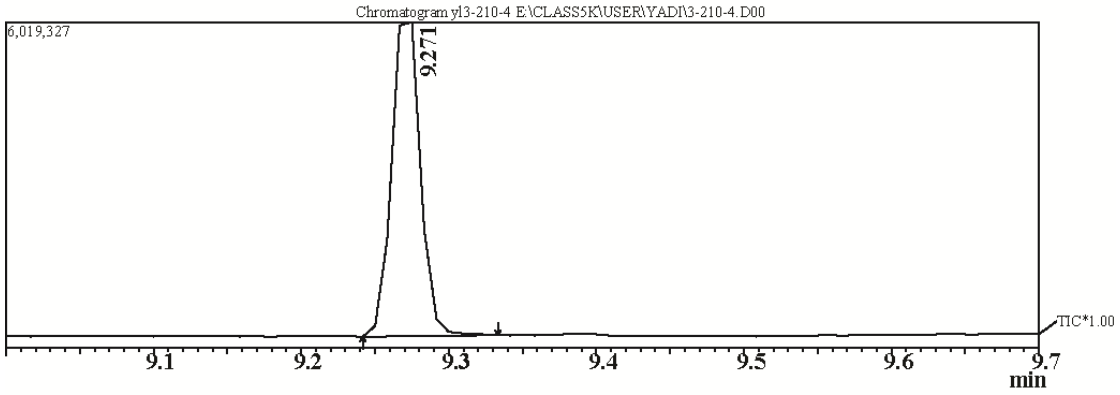
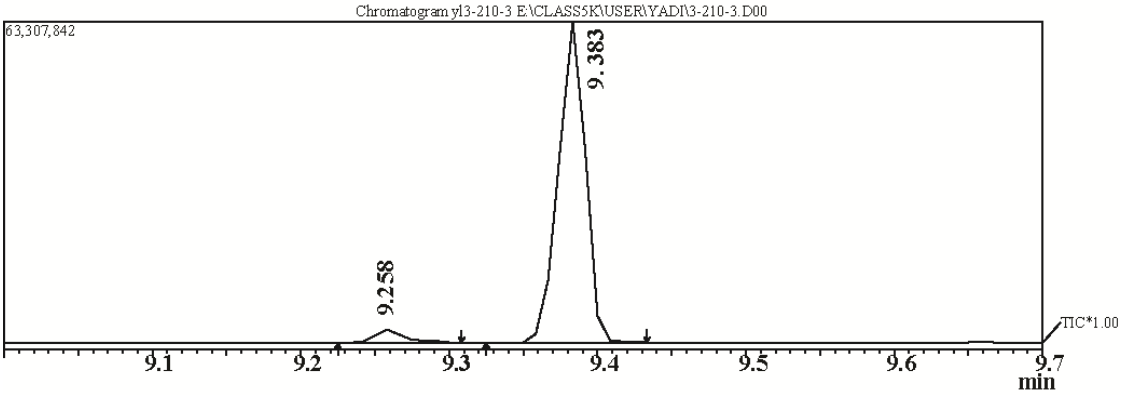
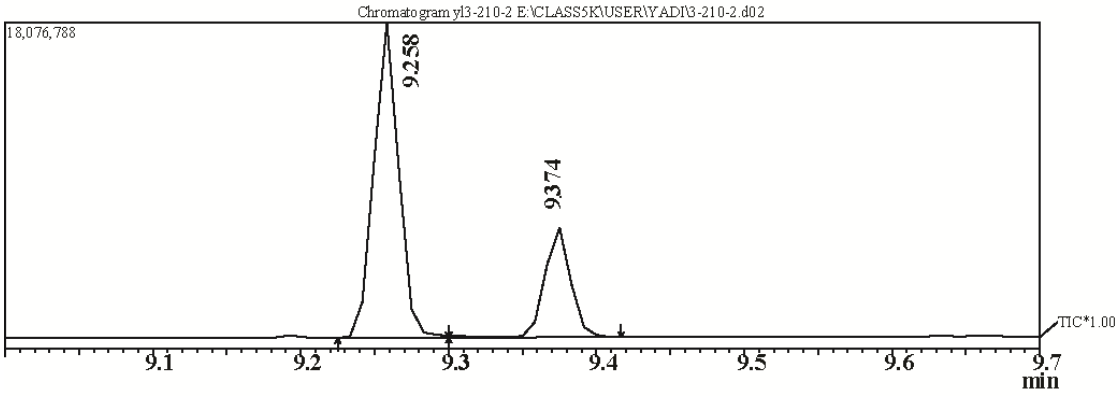
	GC
2.2	
2.3	
2.1	

Table 2.1 Gas chromatography of *cis*, *trans* isomers and their mixture

The *cis* and *trans* aldehyde isomers can be separated on GC/MS. Since the *cis* isomer has already been verified, comparing the GC/MS of compounds **2.1**, **2.2** and **2.3** confirmed that compound **2.3** is the *trans* isomer (Table **2.1**).

2.2.3 Synthesis of 2-(spiro[4,5]decan-8-yl)acetaldehyde and 2-(4,4-diethylcyclohexyl)acetaldehyde (compound 2.4 and 2.5)

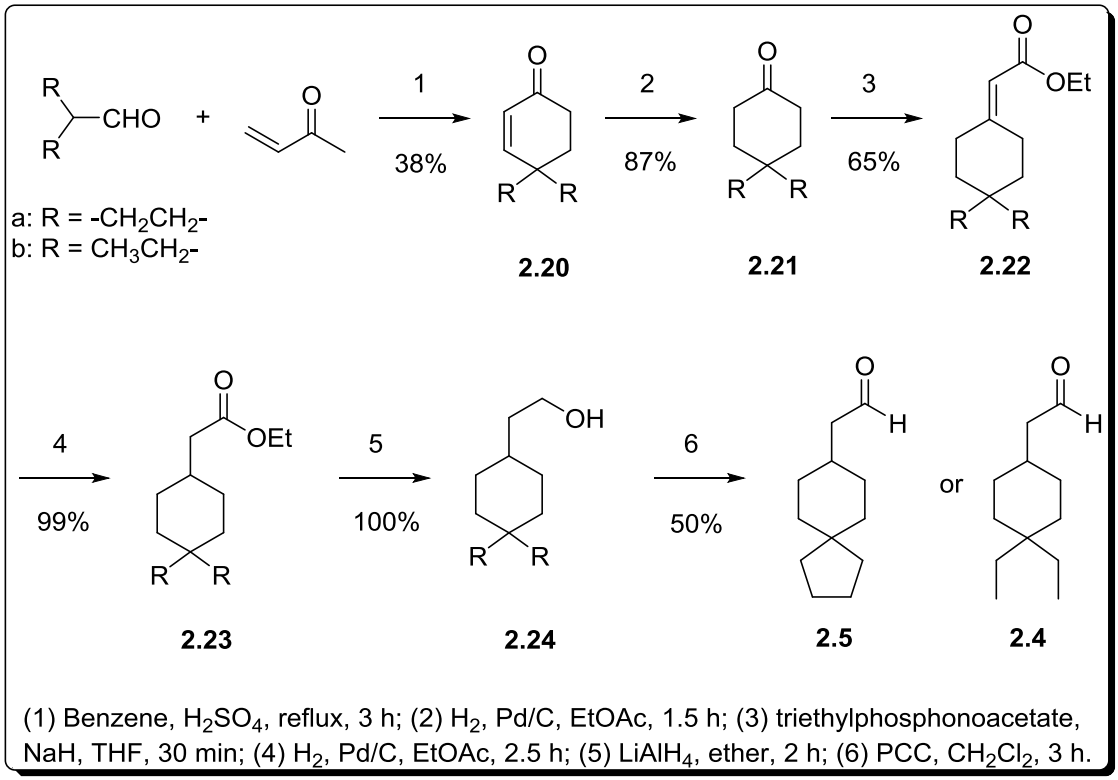


Figure 2.5 Synthesis of 2-(spiro[4,5]decan-8-yl)acetaldehyde and 2-(4,4-diethylcyclohexyl)acetaldehyde

According to literature procedure[4] a Michael-type addition of cyclopentylmethanal and methyl vinyl ketone followed by an aldol-cyclization afforded the spiro enone **2.20**. The α,β-unsaturated ester **2.22** was prepared from **2.20** by hydrogenation and Horner-Wadsworth-Emmons reaction. Hydrogenation of **2.22** gave ester **2.23**. The ester **2.23** was then converted to aldehyde **2.4** and **2.5** by LAH reduction and PCC oxidation. The alcohol **2.24b** was prepared by two former group members with my help (Raul Daniels and Roxane Vabre).

2.2.4 Synthesis of 2-(4-alkylidenecyclohexyl)acetaldehyde

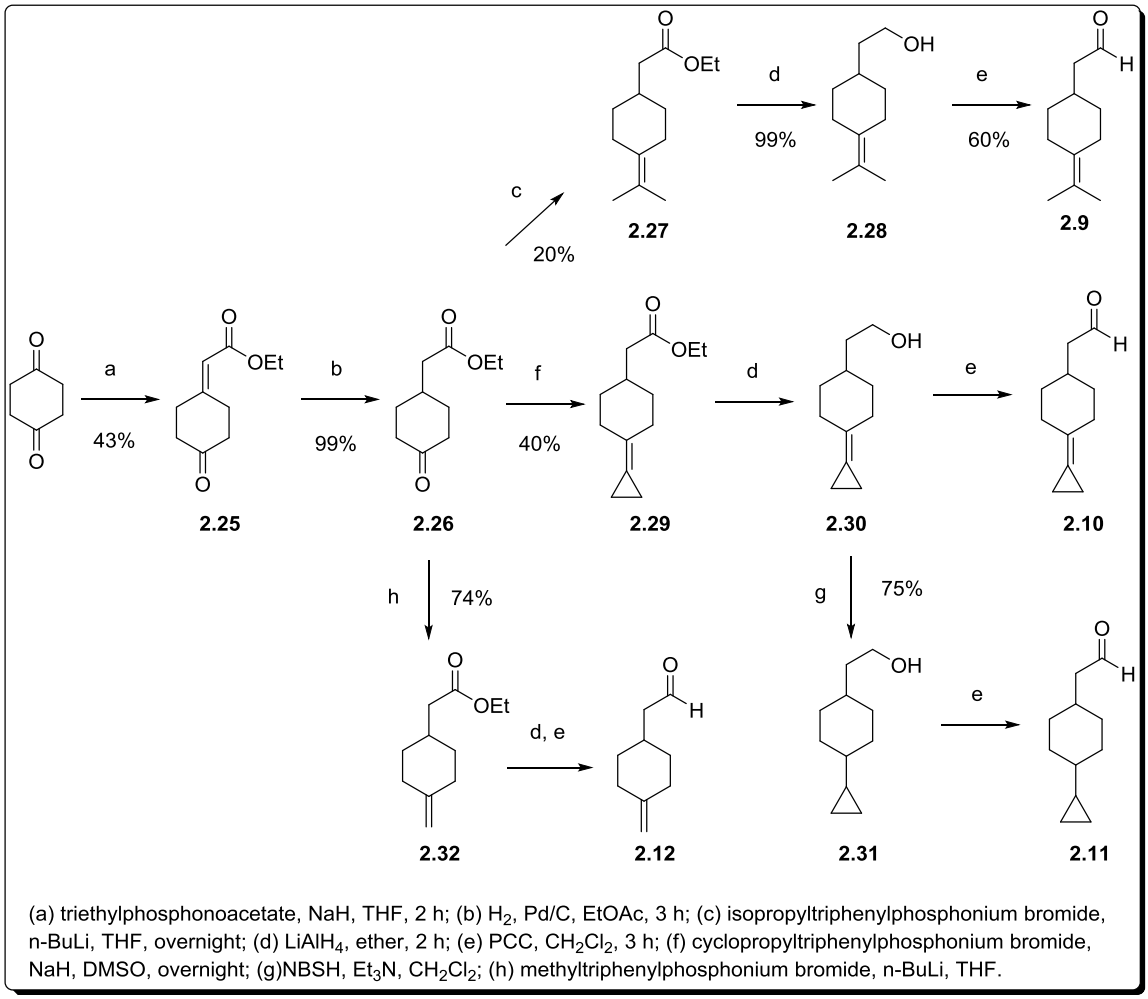


Figure 2.6 Synthesis of 2-(4-(propan-2-ylidene)cyclohexyl)acetaldehyde, 2-(4-cyclopropylidenecyclohexyl)acetaldehyde and 2-(4-cyclopropylcyclohexyl)acetaldehyde

Since the compounds in group 2 share the same cyclohexylacetaldehyde structure fragment, ethyl 2-(4-oxocyclohexyl) acetate (**2.26**) was used as a common intermediate. It was synthesized from 1,4-cyclohexadione by a Honor-Wadsworth-Emmons reaction followed by hydrogenation.

It's generally considered hard to prepare tetra substituted alkenes by Wittig reaction[5]. For synthesis of alkene **2.27**, different conditions and different intermediates were tried, and the yields were poor. The best condition found was to prepare the ylide by using *n*-BuLi in THF and then react with ketone at -40 ºC, giving product **2.27** in ~20% yield. The unreacted ketone can be recovered from chromatography. Similarly, the Wittig reaction of **2.26** with

cyclopropylidenetriphenylphosphorane in THF gave low conversion. But when ylide was prepared in DMSO using NaH as base[6], alkene **2.29** was prepared in 40% yield. The synthesis of alkene **2.32**, on the other hand, worked well under standard condition (*n*-BuLi, THF).

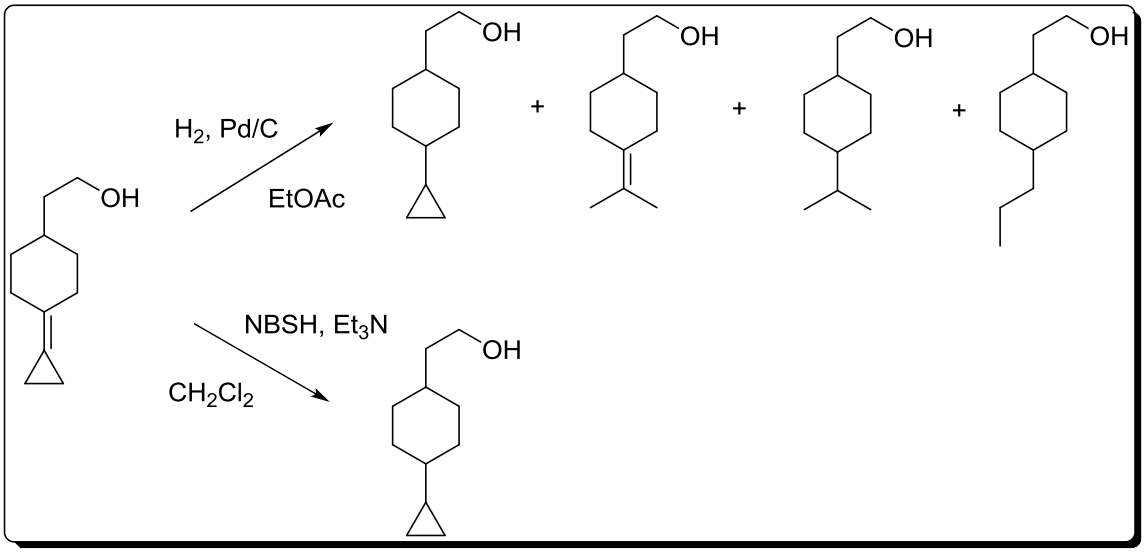


Figure 2.7 Hydrogenation of 2-(4-cyclopropylidenecyclohexyl)ethan-1-ol (2.30)

Direct hydrogenation of cyclopropylidene **2.30** resulted in mixture of cyclopropyl product **2.31** and undesired product**s** (Figure 2.7). *o*-Nitrobenzenesulfonylhydrazide (NBSH)[7] in polar solvent generates diimide which can efficiently reduce alkenes without alkene isomerization and epimerization. Applying NBSH, the C=C double bond of **2.30** was selectively reduced without opening the cyclopropane ring.

The esters and alcohols were converted to final aldehydes by LAH reduction and PCC oxidation.

2.2.5 Synthesis of 2-(4-ethylidenecyclohexyl)acetaldehyde

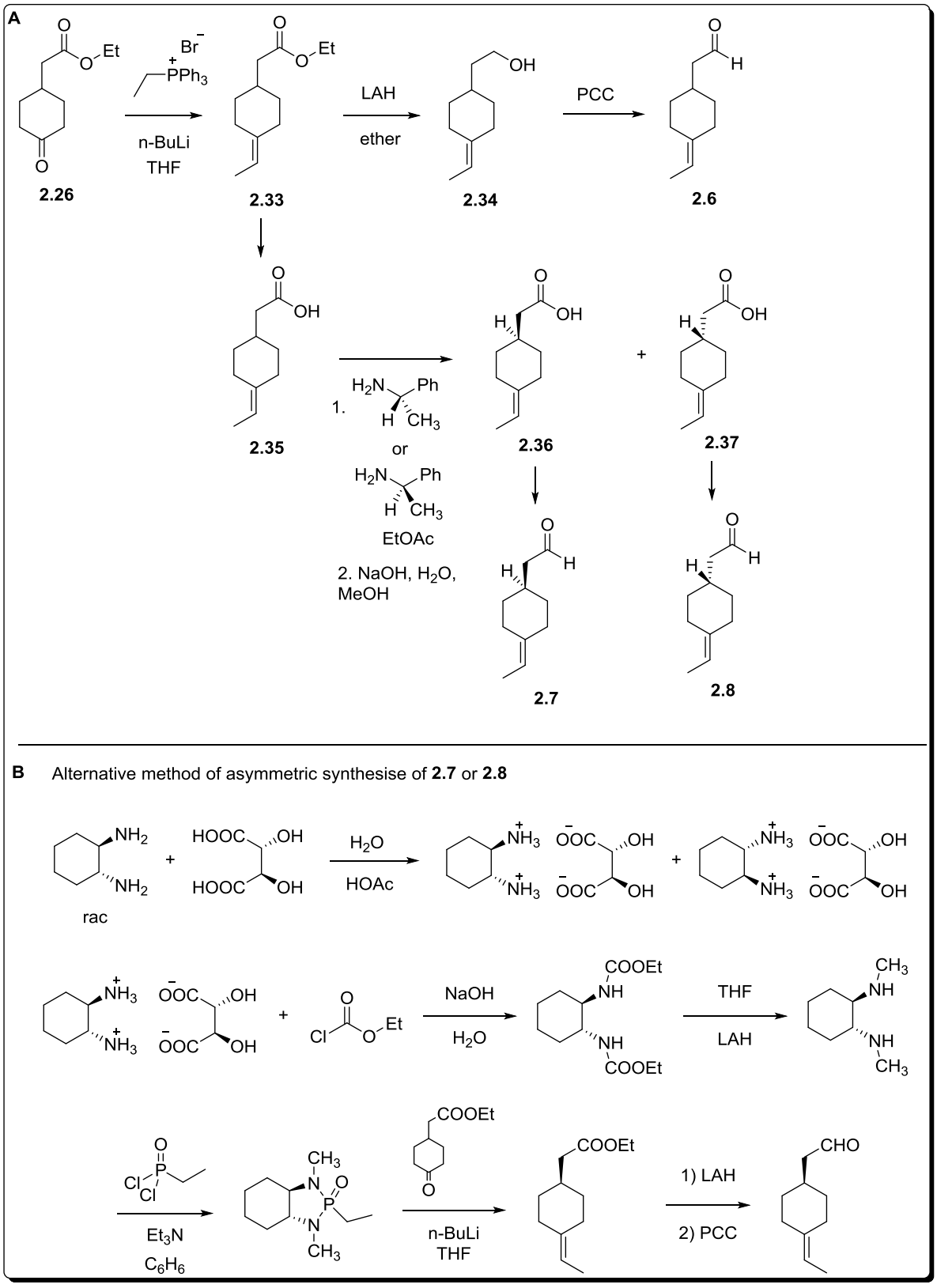


Figure 2.8 Synthesis of 2-(4-ethylidenecyclohexyl)acetaldehyde

The rac-2-(4-ethylidenecyclohexyl)acetaldehyde(**2.6**) was synthesized using the same method as above. The resolution of the racemic acid was done according to literature procedure[8]. Racemic acid **2.35** was mixed with (+) or (-)-α-phenylethylamine and recrystallized from ethyl acetate twice. The resulting salt was decomposed with NaOH and acidified to afford resolved acid. They were then converted to corresponding aldehydes **2.7** and **2.8**.

An asymmetric synthesis of **2.7** and **2.8** using a chiral auxiliary was also tried (Figure 2.7). Racemic *trans*-1,2-diaminocyclohexane was resolved by crystallization with tartaric acid. The (R,R)-1,2-diaminocyclohexane and (S,S)-1,2-diaminocyclohexane were then converted to chiral Wittig reagent according to the procedure by Bennani and Hanessian[9]. Wittig reaction of ethyl 2-(4-oxocyclohexyl)acetate (**2.26**) gives R and S ester that can be converted to **2.7** and **2.8**.

Sample	Amine used for crystallization	m.p. after 1st crystallization	m.p. after 2nd crystallization	Specific rotation
2.36	(+)-methylbenzylamine	88-90 °C	89-91 °C	-2.3 °
2.37	(-)-methylbenzylamine	87-90 °C	88-91 °C	1.6 °C

Table 2.2 Chiral resolution of 2-(4-ethylidenecyclohexyl)acetic acid

The determination of ee % was found to be difficult. There is no reported specific rotation data of the ester/alcohol/aldehyde, so we couldn't calculate the ee % with the specific rotation. Other methods of measuring ee% were also tried, such as: making diastereomeric derivatives (with chiral amine (+)-α-methylbenzylamine or alcohol (-)-menthol) for chromatography separation (GC or LC or TLC), NMR with chiral shift reagent. But we couldn't find a condition to separate the two isomers to calculate the optical purity. As a result, we chose the first resolution method, because the narrow melting point of the recrystallized ammonia salt suggests the product is relatively pure.

2.3 Test result: OR-I7 Activation by Conformationally Restricted Mimics of Octanal

2.3.1 OR-I7 prefers the *trans* isomer over the *cis* isomer

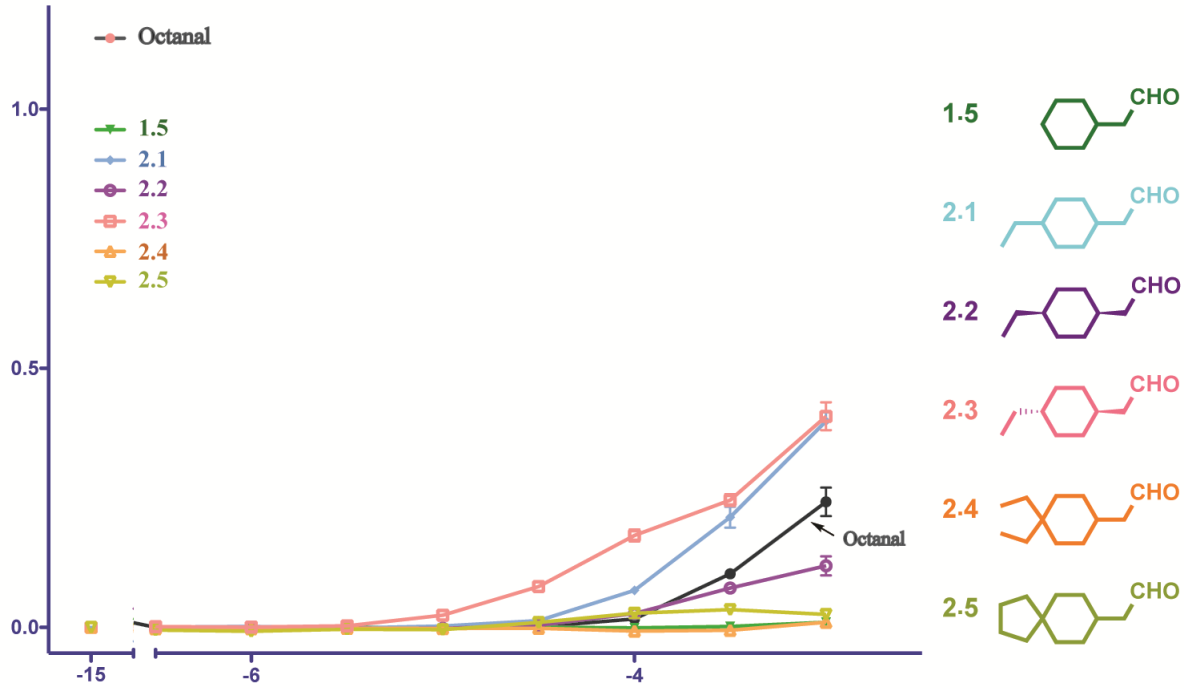


Figure 2.9 Activation dose-response curves of group 1 compounds (Data courtesy of Jianghai Ho, Duke University)

The compounds were tested by Jianghai Ho in Prof. Matsunami's lab at Duke University Department of Molecular Genetics and Microbiology. The activation of OR-I7 by the compounds was measured in Hana3A cells that heterologously expressed OR-I7. When a compound binds and activates the receptor, it triggers cascade events inside the cell including a step of generating cAMP. A cAMP response element binding protein (CREB) is used to track the cAMP. When activated by cAMP, CREB induces the expression of a firefly luciferase reporter gene. The firefly luciferase luminescence was measured and compared with a control *Renilla* luciferase luminescence[10].

The activation responses of the group 1 compounds are shown in Figure **2.8.** As what we have previously seen, the *cis*/*trans* mixture **2.1** showed to be slightly more potent than octanal, confirming the new system is working. The *cis* isomer **2.2** is approximately as potent as octanal; only at high concentration ($>10^{-4}$M) octanal activate more receptors than the *cis* isomer **2.2**. On the other hand, the *trans* isomer **2.3** was more potent than octanal at all concentration tested. We take this result to mean that the *trans* isomer, more than the *cis* isomer, resembles the activating conformation of octanal. We propose that it gains potency by losing less entropy during binding and activation. (The entropy cost was paid during the chemical synthesis of the conformationally restricted analog.)

In other words, the ethyl group in the *trans* isomer is in equatorial position making the molecule a little more extended, while the *cis* isomer has a shorter length with the ethyl group in axial position. The flexibility of C_6-C_7 bond rotation still leads to slightly different length of the molecule and the position of C_8 methyl. Nevertheless, the higher potency of *trans* isomer suggest that the C_5-C_6 bond conformation in *trans* isomer works better in guiding the ethyl group to reach the small "activation pocket" we proposed to explain the potency of the cyclopropyl group in Chapter 1.

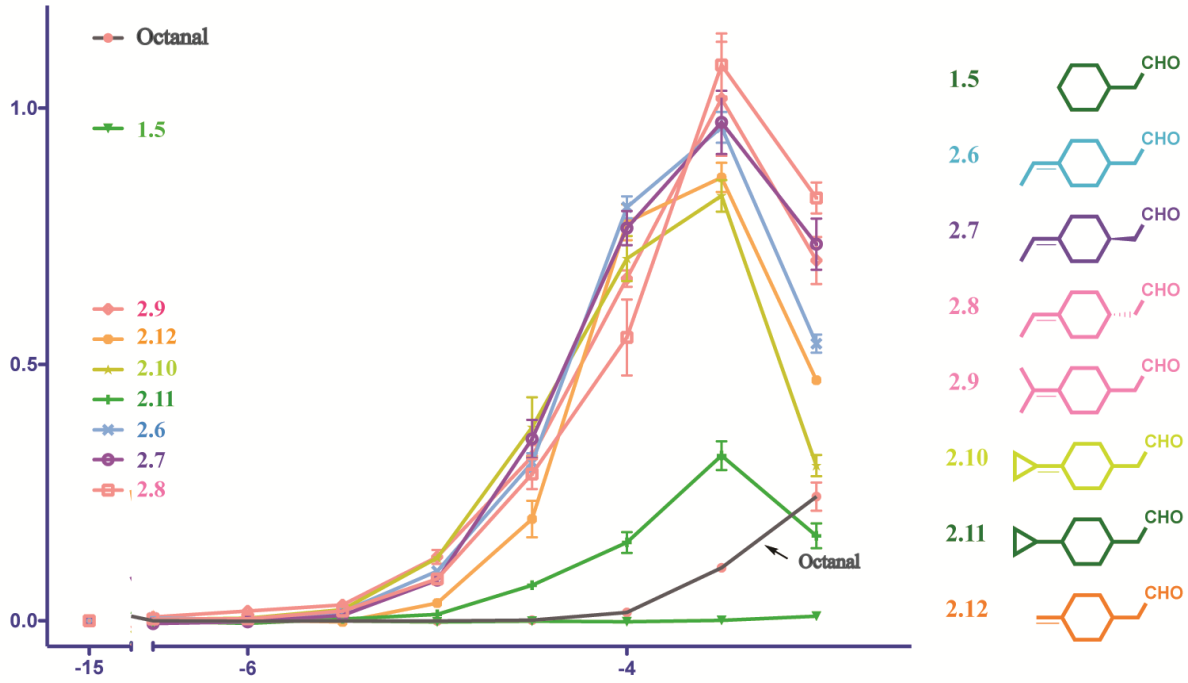


Figure 2.10 Activation dose-responses curves of group 2 compounds (Data courtesy of Jianghai Ho, Duke University)

2.3.2 C_6-C_7 alkene bond enhances OR-I7 activation

The activation responses of the group 2 compounds are shown in Figure **2.10**. Although we have seen compounds that are more potent than octanal (such as compound **2** and **11** in chapter 1), the efficacies of those compounds were comparable to octanal. We were surprised to find out that most of the compounds in group 2 were not only more potent than octanal, but were also more efficacious than octanal (approximately 3-4 times). All these compounds have a C_6-C_7 C=C double bond, suggesting that instead of saturated alkyl chain (like octanal) OR-I7 may actually prefer double bond structure at C_6-C_7 position. Furthermore, the potencies and efficacies of these compounds were close to each other, no matter where is the C_8 groups (compound **2.6**, **2.7**, **2.8**)

or how many of C_8 groups are present (0: compound **2.12**; 1: compound **2.6**, **2.7**, **2.8**; 2: compound **2.9, 2.10**).

The only compound in this group that was relatively less potent is compound **2.11** which doesn't have the C=C bond between C_6 and C_7. It resembles conformation closer to the compounds in the first group. Indeed, compound **2.11** (*cis*/*trans* mixture) showed approximately same potency as compound **2.1** (*cis*/*trans* mixture).

2.3.3 Size of the binding pocket

Although the binding pocket is shown to tolerate some variations, at least for an extra carbon as compound **2.9** and **2.10**, larger groups seem not to be able to fit into the binding pocket. In group 1, both *cis* and *trans* isomers were able to activate OR-I7. When both C_7-C_8 ethyl groups in *cis* and *trans* isomers are present in the structure at the same time, compound **2.4** and **2.5** turned out to lose activity. The free rotation of the two C_6-C_7 bonds in compound **2.4** covers large space and the repulsion between the C_8 methyl groups makes them favor point away from each other. This steric bulkiness is probably the reason that compound **2.4** is completely inactive. When the two C_8 carbons are brought close by a C-C bond, compound **2.5** shows weak activity. These results suggest that there is some steric limitation around the activation pocket.

2.4 Discussion

The lengths of compounds range from 6.6 to 8.0 Å (**Table 2.1**), which is longer than the previously determined minimum length for activation (6.5-6.9 Å)[1] and shorter than the extended length of octanal (8.9 Å). The length of the molecule should not be the main determinant of the potency of the compound. Since most of the compounds have little flexibility, the lengths of the compounds are likely the real distance between C_1 and the end carbon when they bind to OR-I7. Analyzing the relationship between the structure of these compounds and their activity can help

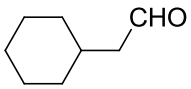
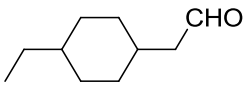
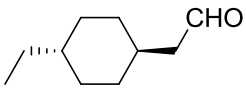
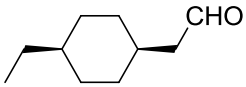
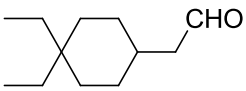
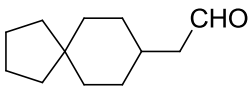
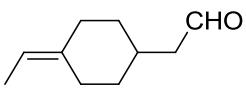
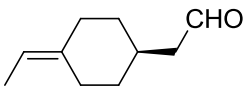
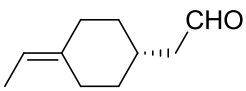
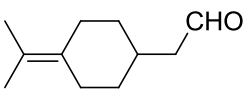
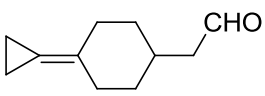
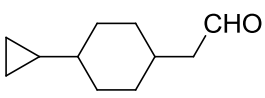
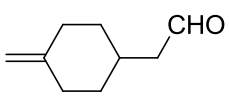
Compound	Structure	Sample number	Length
1.5		Yl3-210-1	5.56
2.1		Yl3-210-2	7.30 and 7.95
2.2		Yl3-210-4	7.95
2.3		Yl3-210-3	7.30
2.4		Yl3-210-5	7.69
2.5		Yl3-210-6	7.90
2.6		Yl3-210-11	7.67
2.7		Yl3-210-12	7.67
2.8		Yl3-210-13	7.67
2.9		Yl3-210-7	7.61
2.10		Yl3-210-9	7.82
2.11		Yl3-210-10	8.01
2.12		Yl3-210-8	6.55

Table 2.3 Length of conformational analogues of octanal

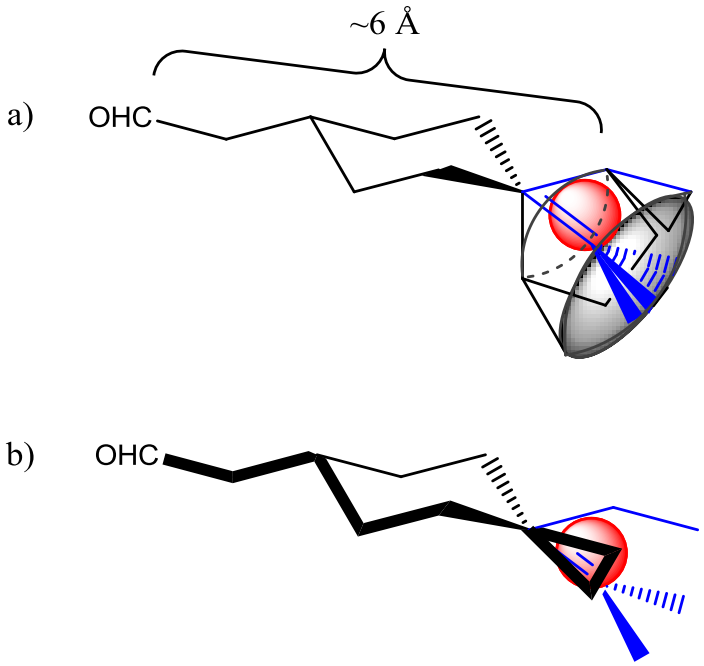


Figure 2.11 Model of activation binding pocket of OR-I7

us to obtain more information about the "activation pocket". To compare different conformations of the compounds, their structures are stacked together based on the same cyclohexylacetaldehyde (**Figure 2.11**). The more active conformations are marked with blue color.

Most of the compounds tested turned out to be equal or more potent and efficacious than octanal. The two exceptions (compounds **2.9** and **2.10)** were inactive because of their steric bulkiness rather than conformation. The common structure they share: gauche conformation at C_4-C_5 must contribute to the high activity within these compounds. Because of the restriction of the cyclohexyl ring, the tails of the compounds are guided to a relatively limited region (gray region).

The conformation of C_6-C_7 bond seems to be important for the activity. The compounds having a C=C bond at C_6-C_7 position forms a planar structure around C_6 and C_7. The space between this plane and the C_6-C_7 bond of the *cis* isomer (red area in **Figure 2.10a**) forms a highly active

"neck" that all the more potent compounds pass through with their C_6-C_7 bond. Compound **2.4** cannot squeeze two C_6-C_7 bonds through this neck and cannot activate OR-I7. For compound **2.5**, the two C_6-C_7 bonds are brought slightly closer by the conformational restriction of the ring, which makes it an agonist but weak. Using this model we can explain the high potency of the cyclopropyl compound (compound **1.2**) in Chapter 1. If we align the chain of compound **1.2** to the model, then the cyclopropyl ring passes through the red area (**Figure 2.10b**).

There is a possible structural explanation for this requirement of C_6-C_7 bond conformation. Computational modeling study by our collaborators revealed that two amino acid residues F205 and Y264 containing benzene ring form hydrophobic interaction with the tail of carbon chain and are responsible for the size of the binding pocket[11]. It was also proposed that the conformation of F205 is essential for maintaining the hydrogen bond of the aldehyde with the receptor. The planar structure of sp2 C_6 and C_7 or the cyclopropyl ring probably help to better interact with the benzene ring on amino acid.

Lastly, once the C_6-C_7 bond passes through the red "neck", the carbons, including the second carbon of the double bond, C_7, and beyond C_7 are inside the activation pocket. The activation pocket[1] can tolerate different conformations of small alkyl groups. Compounds with the same C_6-C_7 conformation having one C_8 (**2.6**, **2.7**, **2.8**), or two C_8 (**2.9**), or even without C_8 (**2.12**) all have approximately the same potencies. The two cyclopropyl ring containing compound **2.10** and **2.11** did not show significant increase in potency as compared to their corresponding analogs (**2.6** and **2.1)** without the cyclopropyl ring. Since both cyclopropyl rings are inside the activation pocket, **2.10** and **2.11** provide two more examples to support that the activation pocket can tolerate some variations.

2.5 Experimental Procedures

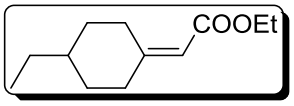
Materials

Commercial reagents and solvents were used without additional purification unless otherwise noted. THF and ether were dried and distilled from Na/benzophenone. All other reagents were from Sigma-Aldrich or Alfa Aesar. Reaction progress and chromatography fractions were monitored by thin layer chromatography (TLC) on silica gel coated glass plates. Flash chromatography purifications[12] were performed using 230-400 mesh silica gel from Alfa Aesar. Freshly purified aldehydes were stored at 4 ºC under Argon and tested within 1 week.

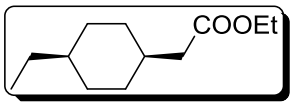
Compound characterization

NMR spectra were recorded on a Varian Mercury-300 spectrometer. Chemical shifts are reported in parts per million (ppm) referenced to the appropriate solvent peak. Infrared (IR) spectra were recorded using a Thermo Nicolet 6700 FT-IR spectrometer, and are reported in wavenumbers (cm^{-1}). GC/MS analyses were obtained on (1). Shimadzu GC/MS QP5000 with GC-17A Gas Chromatograph (capillary column: DB-1-30N-STD). Temperature programs for GC analysis: Program 1: Injection temperature: 280 °C; held at 60 °C for 4 min, heated from 60 to 280 °C at 10 °C/min, and held at 280 °C for 2 min; Program 2: Injection temperature: 280 °C; held at 60 °C for 4 min, heated from 60 to 280 °C at 20 °C/min, and held at 280 °C for 2 min; Program 3: Injection temperature: 280 °C; held at 60 °C for 1 min, heated from 60 to 280 °C at 20 °C/min, and held at 280 °C for 5 min. (2) Shimadzu GCMS-QP2010 with GC-2010 Plus Gas Chromatograph. Program 4: Injection temperature: 280 °C; held at 40 °C for 4 min, heated from 40 to 200 °C at 15 °C/min, and held at 200 °C for 3 min; Program 5: Injection temperature:

280 °C; held at 40 °C for 4 min, heated from 40 to 200 °C at 10 °C/min, and held at 200 °C for 4 min.



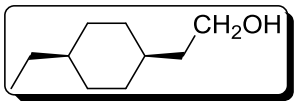
Ethyl 2-(4-ethylcyclohexylidene)acetate (2.13)[1, 13] (compound **12** in chaptor 1)**:** Ethyl 2-(diethoxyphosphoryl)acetate (8.88 g, 39.6 mmol) in 15 mL dry THF was added dropwise to NaH (washed with pentane to remove oil) in 50 mL THF. After the addition, the reaction mixture was stirred for 1 h at room temperature. 4-ethylcyclohexanone (5 g, 39.6 mmol) in 10 mL dry THF was added at a rate that the temperature of the mixture was kept below 30 °C. The solution was then stirred for 15 min during which time a viscous semi-solid appeared. The mixture was taken up in a large excess of water and the aqueous solution was extracted with ether. The ether layer was dried and concentrated. The crude product was purified by column chromatography using ether/hexane (1:19) to give colorless liquid **2.13** (6.84 g, 88%). IR (thin film, NaBr plates), ν: 2933, 2855, 1716, 1650, 1186, 1150; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.58 (s, 1H), 4.12 (q, *J* = 7.1, 2H), 3.73 (d, *J* = 14.2, 1H), 2.34–2.03 (m, 2H), 2.01–1.79 (m, 3H), 1.46–1.31 (m, 1H), 1.30–1.14 (m, 5H), 1.12–0.95 (m, 2H), 0.87 (t, *J* = 7.4, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.0, 163.8, 113.1, 59.6, 39.1, 37.5, 34.3, 33.6, 29.1, 14.5, 11.8; MS (EI): 196 (M+).



Ethyl (*cis*-4-ethylcyclohexyl)acetate (2.14)[3b]: Ethyl 2-(4-ethylcyclohexylidene)acetate (0.5g, 2.55 mmol) in dry THF (2.5 mL) was added slowly to 1 M L-selectride in THF (7.64 mL, 7.64 mmol), keeping the temperature below -70 °C. The mixture was stirred at -75 °C for 1 h, and at -40 °C for 4h. Ethanol (1.2 mL) was added followed by EtOAc (3.6 mL), and pH=7 phosphate buffer (0.1 M, 1.6 mL). 30% H_2O_2 solution (2.2 mL) was then added slowly. The mixture was stirred overnight before partitioning between EtOAc and H_2O. The aqueous layer was extracted with EtOAc. The combined organic

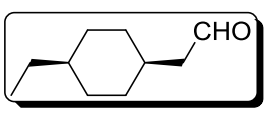
layers were extracted with brine, dried and concentrated. The crude product was chromatographed with 2% ether and 98% hexane to give 0.22 g (44%) colorless liquid **2.14**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.12 (q, *J* = 7.1 Hz, 2H), 2.27 (d, *J* = 7.5 Hz, 2H), 1.54–1.43 (m, 4H), 1.43–1.15 (m, 11H), 0.86 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 173.5, 60.1, 39.2, 37.0, 32.5, 28.7, 28.2, 26.9, 14.4, 14.2, 12.0, 11.7; IR (neat), ν: 2960, 2920, 2854, 1735, 1451, 1166, 1034; GC retention time: 11.91 min (method: program 1); MS (EI): 198 (M+).

General Procedure for Lithium Aluminum Hydride (LAH) Reduction:



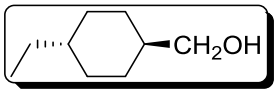
2-(*cis*-4-ethylcyclohexyl)ethanol (2.15): To an ice-cold mixture of LAH (0.122 g, 3.20 mmol) in 6 mL dry ether under N_2, was added dropwise **2.14** (0.53 g, 2.67 mmol) in 3 mL dry ether. The mixture was stirred at room temperature for 3 h then re-cooled to 0° C. The mixture was worked up by dropwise and sequential addition of 0.12 ml H_2O, 0.12 ml 15% NaOH solution and 0.36 ml H_2O. After stirring for few minutes, the mixture was filtered through a celite pad and washed with ether. The solution was dried and concentrated under reduced pressure. The colorless liquid product was used without further purification. Yield: 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.65 (t, *J* = 6.7 Hz, 2H), 1.59–1.38 (m, 7H), 1.38–1.22 (m, 8H), 0.84 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 61.2, 37.2, 37.1, 31.7, 28.9, 28.4, 26.9, 11.8; IR (neat), ν: 3320 (broad), 2958, 2915, 2872, 2851, 1449, 1377, 1061, 1010, 978; GC retention time: 9.98 min (method: program 2); MS (EI): 138 $(M\text{-}H_2O)^{+}$.

General Procedure for Pyridinium Chlorochromate (PCC) Oxidation:

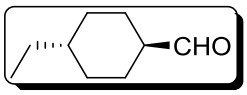


2-(*cis*-4-ethylcyclohexly)acetaldehyde (2.3): To a stirred suspension

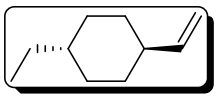
of PCC (0.62 g, 2.88 mmol) and silica gel (0.62 g) in CH_2Cl_2 (7 mL) was added a solution of the alcohol (0.3 g, 1.92 mmol) in 3 mL CH_2Cl_2. The mixture was stirred at room temperature for 2 h and then filtered through a silica gel pad which was rinsed thoroughly with fresh solvent. The solvent was evaporated and the crude product was purified by column chromatography with ether/hexane (1:19) to give colorless liquid **2.3**. Yield: 60%. ^{1}H NMR (300MHz ,$CDCl_3$) δ 9.75 (t, *J* = 2.2 Hz, 1 H), 2.38 (dd, *J* = 2.3, 7.0 Hz, 2 H), 2.26 - 2.06 (m, 1 H), 1.66 - 1.45 (m, 4 H), 1.45 - 1.16 (m, 7 H), 0.86 (t, *J* = 7.0 Hz, 3 H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 203.0, 48.3, 36.9, 30.1, 28.9, 28.2, 27.0, 11.8; IR (neat), ν: 2959, 2920, 2854, 2709, 1725, 1450, 1409, 1378, 1290, 1172, 1097, 998, 932, 890, 812, 660; GC retention time: 9.38 min (method: program 2); MS (EI): 154 (M^+).



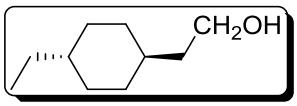
(*trans*-4-ethylcyclohexyl)methanol (2.16)[14]: LAH reduction of (*trans*-4-ethylcyclohexyl)carboxylic acid (5.7 g, 0.05 mol) (purchased from OChem Incorporation) as the general procedure described above to give colorless liquid **2.16**. Yield: 97%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.41 (t, *J* = 5.9 Hz, 2H), 1.76 (d, *J* = 9.3 Hz, 4H), 1.42 (t, *J* = 5.6 Hz, 2H), 1.19 (p, *J* = 6.6 Hz, 2H), 1.05 (s, 1H), 0.85 (m, *J* = 7.2 Hz, 7H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 68.7, 40.6, 39.5, 32.2, 29.9, 29.5, 11.4; IR (neat), ν: 3320, 2959, 2912, 2849, 1447, 1378, 1100, 1071, 1035, 1002, 964, 895; GC retention time: 10.35 min (method: program 4); 124 ($M-H_2O^+$)



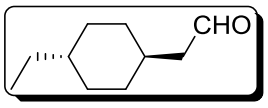
(*trans*-4-ethylcyclohexyl)carbaldehyde (2.17)[14]: PCC oxidation of (*trans*-4-ethylcyclohexyl)methanol **2.16** (3 g, 0.021 mol) as the general procedure described above to give colorless liquid **2.17**. Crude yield: 91%. It was noticed that the product was very easily oxidized when exposed to the air. The freshly prepared aldehyde was immediately used for the next reaction without column chromatography.



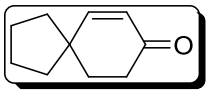
***trans*-1-ethyl-4-vinylcyclohexane (2.18):** To an ice-cooled mixture of methyl triphenylphosphonium bromide (10.33 g, 0.029 mol) in dry THF (75 mL) was added 1.6 M *n*-BuLi solution (16.9 mL, 0.027 mol). The mixture was stirred at 0 °C for 30 min, then cooled to -75 °C. (*trans*-4-ethylcyclohexyl)carbaldehyde **2.17** (2.7 g, 0.019 mol) in THF (25 mL) was added dropwise. The mixture was stirred at -75 °C for 3 h. NH_4Cl solution was added and the layers were separated. The organic layers were extracted with H_2O, brine and dried. The crude product was chromatographed with pentane to give 2.5 g (95%) colorless liquid **2.18**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.76 (ddd, *J* = 17.1, 10.4, 6.5 Hz, 1H), 5.00–4.80 (m, 2H), 2.06–1.60 (m, 3H), 1.20 (m, 3H), 1.03 (m, 3H), 0.97–0.77 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.08, 111.77, 42.13, 39.29, 32.70 (d, *J* = 2.1 Hz), 31.82, 30.21, 22.89, 14.37, 11.75; GC retention time: 6.29 min (method: program 1); MS (EI): 138 (M^+).



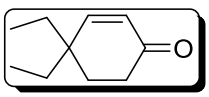
2-(*trans*-4-ethylcyclohexyl)ethanol (2.19): 1 M BH_3 (9 mL, 0.009 mol) in THF was added dropwise to a solution of *trans*-1-ethyl-4-vinylcyclohexane **2.18** (2.5 g, 0.018 mol) in THF (50 mL). After stirred for 15 min, saturated Na_2CO_3 solution (6.2 mL) and 30% H_2O_2 solution (3.68 mL, 0.036 mmol)) was added. The mixture was stirred for 2 h. H_2O (50 mL) was added and the layers were separated. The aqueous layer was extracted with ether. The combined organic layers were washed with brine, dried and concentrated. The crude product was chromatographed with 5% acetonitrile, 45% CH_2Cl_2 and 50% cyclohexane to give 1.2 g (43%) colorless liquid **2.19**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.66 (t, *J* = 5.5 Hz, 2H), 1.72 (d, *J* = 8.9 Hz, 4H), 1.51–1.37 (m, 3H), 1.37-1.25 (m, 1H), 1.25–1.11 (m, 3H), 1.10-0.99 (m, 1H), 0.98–0.75 (m, 6H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 60.8, 40.3, 39.4, 34.4, 33.2, 32.7, 29.9, 11.4; IR (neat), ν: 3321, 2959, 2912, 2849, 1447, 1378, 1045, 1021, 1002, 977, 895; GC retention time: 9.86 min (method: program 2); MS (EI): 138 $(M\text{-}H_2O)^+$.



2-(*trans*-4-ethylcyclohexyl)acetaldehyde (2.2): PCC oxidation of 2-(*trans*-4-ethylcyclohexyl)ethanol **2.19** (0.3 g, 1.92 mmol) as the general procedure described above to give colorless liquid **2.2**. Yield: 57%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.73 (t, *J* = 2.3 Hz, 1H), 2.27 (dd, *J* = 6.7, 2.3 Hz, 2H), 1.86–1.68 (m, 5H), 1.18 (p, *J* = 7.5, 6.9 Hz, 2H), 1.07–0.78 (m, 8H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 202.9, 51.3, 39.0, 33.1, 32.9, 32.5, 29.8, 11.4; IR (neat), ν: 2960, 2914, 2849, 2711, 1724, 1447, 1378, 1218, 1150, 1025, 897; GC retention time: 9.27 min (method: program 2); MS (EI): 154 (M^+).

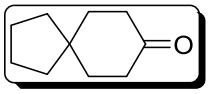


Spiro[4,5]dec-6-en-8-one (2.20a)[4, 15]: Freshly prepared cyclopentylcarbaldehyde (2.3 g, 23.5 mmol, from PCC oxidation of cyclopentylmethanol) and methyl vinyl ketone (1.83g, 23 mmol) were dissolved in benzene (20 mL) in a flask connected to a Dean-stark trap. 0.02 mL concentrated H_2SO_4 was added. The mixture was slowly heated to boiling over 1 h and then kept refluxing for 3 h. The cooled mixture was washed with $NaHCO_3$ solution. The aqueous layer was extracted with ether. The combined organic layers were dried ($MgSO_4$) and concentrated. The crude product was chromatographed with 10% EtOAc, 90% hexane, to give 1.3 g (38%) colorless liquid **2.20a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.71 (d, *J* = 10.1 Hz, 1H), 5.82 (d, *J* = 10.1 Hz, 1H), 2.41 (t, *J* = 6.7 Hz, 2H), 1.87 (t, *J* = 6.7 Hz, 2H), 1.77–1.55 (m, 8H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 200.1, 159.7, 126.5, 44.1, 38.0, 35.3, 33.9, 33.8, 33.8, 24.5; IR (neat), ν: 2949, 2862, 1672, 1447, 1417, 1389, 1229, 1138, 992, 924, 792, 756, 730; GC retention time: 10.83 min (method: program 1); MS (EI): 150 $(M)^+$.

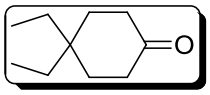


4,4-diethylcyclohex-2-enone (2.20b)[16]: Prepare from methyl vinylketone and 2-ethylbutanal by the same procedure as **2.20a**. Spectral data match those reported previously[16].

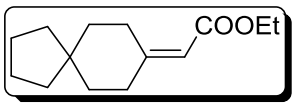
General procedure of hydrogenation:



Spiro[4,5]decan-8-one (2.21a): A mixture of Spiro[4,5]dec-6-en-8-one (0.7g, 4.67 mmol) and Pd/C (0.07g) in EtOAc (30 mL) was stirred under H_2 (balloon) for 1.5h. The mixture was then filtered through celite. EtOAc was evaporated to give 0.6 g (87%) colorless liquid **2.21a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.31 (t, *J* = 6.9 Hz, 4H), 1.72 (t, *J* = 6.9 Hz, 4H), 1.66 (dt, *J* = 6.8, 3.0 Hz, 4H), 1.53 (t, *J* = 5.4 Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 213.14, 42.09, 39.32, 37.90, 37.39, 24.69; IR (neat), ν: 2947, 2856, 1715, 1446, 1336, 1230, 1143, 962, 760; GC retention time: 10.45 min (method: program 1); MS (EI): 152 $(M)^+$.

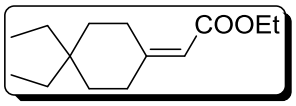


4,4-diethylcyclohexanone (2.21b): Prepared from 4,4-diethylcyclohex-2-enone **2.20b** by the same procedure as **2.21a**. ^{1}H NMR (300MHz ,$CDCl_3$) δ 2.29 (t, *J* = 6.7 Hz, 4 H), 1.63 (t, *J* = 6.7 Hz, 4 H), 1.40 (q, *J* = 7.7 Hz, 4 H), 0.82 (t, *J* = 7.6 Hz, 6 H); IR (neat), ν: 2963, 2026, 2879, 2858, 1714, 1462, 1165, 1137; GC retention time: 11.29 min (method: program 4); MS (EI): 154 $(M)^+$

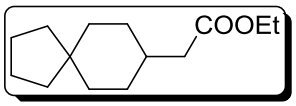


Ethyl 2-(spiro[4,5]decan-8-ylidene)acetate (2.22a): To a suspension of NaH (0.18g, 4.57 mmol, 60% in mineral oil, washed three times with pentane) in dry THF (6 mL) was added triethylphosphonoacetate (1.03g, 4.57 mmol) in THF (2 mL) dropwise. The mixture was stirred at room temperature for 1 h. Spiro[4,5]decan-8-one **2.21a** (0.7 g, 4.57 mmol) in THF was added slowly so that the temperature was kept below 30 °C. The mixture was stirred for 30 min when a viscous precipitate formed. H_2O (10 mL) was added and the two layers were separated. The aqueous layer was extracted with ether. The combined organic layers were dried and concentrated. The crude product was chromatographed with 5% ether , 95% hexane to give 1.13 g (65%) colorless liquid **2.22a**. ^{1}H NMR (300 MHz,

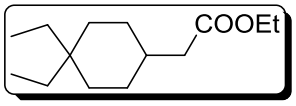
$CDCl_3$) δ 5.58 (s, 1H), 4.11 (q, *J* = 7.1 Hz, 2H), 2.81 (t, *J* = 5.9 Hz, 2H), 2.18 (t, *J* = 5.9 Hz, 2H), 1.66–1.54 (m, 4H), 1.54–1.36 (m, 8H), 1.24 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.07, 164.08, 113.03, 59.67, 42.63, 39.71, 38.92, 37.98, 35.35, 27.01, 24.76, 14.53; IR (neat), ν: 2935, 2852, 1712, 1647, 1446, 1378, 1210, 1165, 1135, 1039, 861; GC retention time: 15.41 min (method: program 1); MS (EI): 222 (M^+).



Ethyl 2-(4,4-diethylcyclohexylidene)acetate (2.22b): Prepared from 4,4-diethylcyclohexanone **2.21b** by the same procedure as **2.22a**. ^{1}H NMR (300MHz ,$CDCl_3$) δ 5.59 (br. s., 1 H), 4.12 (q, *J* = 6.3 Hz, 2 H), 2.81 (t, *J* = 5.8 Hz, 2 H), 2.18 (t, *J* = 6.1 Hz, 2 H), 1.50 - 1.17 (m, 11 H), 0.76 (t, *J* = 6.9 Hz, 6 H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 166.9, 164.1, 112.8, 59.4, 36.3, 35.6, 34.6, 33.1, 27.9, 24.9, 14.3, 7.5; IR (neat), ν: 2965, 2928, 2879, 2655, 1716, 1650, 1461, 1380, 1263, 1200, 1166, 1043, 861; GC retention time: 14.59 min (method: program 1); MS (EI): 224 $(M)^+$.

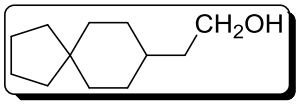


Ethyl 2-(spiro[4,5]decan-8-yl)acetate (2.23a): Hydrogenation of ethyl 2-(spiro[4,5]-8-ylidene)acetate **2.22a** (1 g, 4.5 mmol) as the general procedure described above to give colorless liquid **2.23a**. Yield: 99%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.09 (q, *J* = 7.1 Hz, 2H), 2.15 (d, *J* = 7.1 Hz, 2H), 1.80–1.17 (m, 18H), 1.15–0.95 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.49, 60.30, 42.27, 42.10, 42.03, 37.75, 34.96, 34.86, 30.22, 25.15, 24.28, 14.51; IR (neat), ν: 2916, 2851, 1734, 1446, 1368, 1329, 1286, 1240, 1173, 1124, 1033, 935; GC retention time: 14.65 min (method: program 1); MS (EI): 224 $(M)^+$.

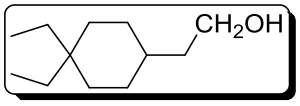


Ethyl 2-(4,4-diethylcyclohexyl)acetate (2.23b): Prepared from Ethyl 2-(4,4-diethylcyclohexylidene)acetate **2.22b** by the same procedure as **2.23a**. ^{1}H NMR (300MHz ,$CDCl_3$) δ 4.10 (q, *J* = 7.0 Hz, 2 H), 2.17 (d, *J* = 6.9 Hz,

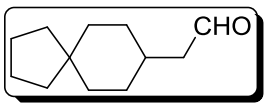
2 H), 1.70 (br. s., 1 H), 1.58 - 1.36 (m, 4 H), 1.36 - 0.96 (m, 11 H), 0.82 - 0.58 (m, 6 H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 173.3, 60.1, 41.9, 35.0, 34.4, 34.1, 32.6, 27.9, 23.7, 14.3, 7.3; IR (neat), ν: 2964, 2922, 2655, 1736, 1462, 1377, 1277, 1243, 1190, 1142, 1034; GC retention time: 14.07 min (method: program 1); MS (EI): 226 $(M)^+$.



2-(spiro[4,5]decan-8-yl)ethanol (2.24a): LAH reduction of ethyl 2-(spiro[4,5]decan-8-yl)acetate **2.23a** (1 g, 4.46 mmol) as the general procedure described above to give colorless liquid **2.24a**. Yield: 100%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.66 (t, *J* = 6.8 Hz, 2H), 1.68–1.14 (m, 18H), 1.11–0.91 (m, 2H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 60.9, 42.4, 41.9, 40.0, 37.8, 34.7, 34.0, 30.3, 24.9, 24.0; IR (neat), ν: 3319, 2913, 2849, 1445, 1048, 1024, 1004, 936; GC retention time: 13.31 min (method: program 1); MS (EI): 182 $(M)^+$, 164 $(M\text{-}H_2O)^+$.

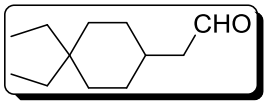


2-(4,4-diethylcyclohexyl)ethanol (2.24b): Prepared from Ethyl 2-(4,4-diethylcyclohexyl)acetate **2.23b** by the same procedure as **2.24a**. ^{1}H NMR (300MHz ,$CDCl_3$) δ 3.65 (t, *J* = 6.7 Hz, 2 H), 1.86 (br. s., 1 H), 1.58 - 1.39 (m, 6 H), 1.31 (q, *J* = 7.6 Hz, 3 H), 1.23 - 0.95 (m, 6 H), 0.83 - 0.62 (m, 6 H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 60.8, 40.0, 34.6, 34.3, 32.7, 28.2, 23.8, 7.3, 7.2; IR (neat), ν: 3321, 2962, 2915, 2852, 1459, 1377, 1062, 1040, 1001, 974, 905, 846, 758; GC retention time: 12.77 min (method: program 1); MS (EI): 155 $(M\text{-}CH_2CH_3)^+$.

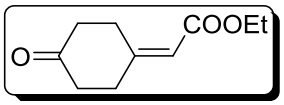


2-(spiro[4,5]decan-8-yl)acetaldehyde (2.5): PCC oxidation of 2(spiro[4,5]decan-8-yl)ethanol **2.24a** (0.3 g 1.65 mmol) as the general procedure described above to give colorless liquid **2.5**. Yield: 50%. ^{1}H NMR (300MHz ,$CDCl_3$) δ 9.76 (t, *J* = 2.5 Hz, 1 H), 2.31 (dd, *J* = 2.5, 6.9 Hz, 2 H), 1.84 (m, *J* = 3.7, 7.3, 11.0 Hz, 1 H),

1.68 - 1.52 (m, 6 H), 1.52 - 1.22 (m, 8 H), 1.20 - 1.02 (m, 2 H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 203.0, 51.0, 42.0, 41.8, 37.5, 34.7, 32.5, 30.2, 24.9, 24.0; IR (neat), ν: 2915, 2850, 2710, 1724, 1446, 1408, 1296, 1186, 1029, 935, 898; GC retention time: 10.98 min (method: program 2); MS (EI): 180 $(M)^+$.

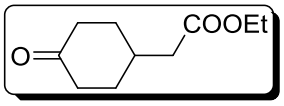


2-(4,4-diethylcyclohexyl)acetaldehyde (2.4): PCC oxidation of 2-(4,4-diethylcyclohexyl)ethanol **2.24b** as the general procedure described above to give colorless liquid **2.4**. ^{1}H NMR (300MHz ,$CDCl_3$) δ 9.75 (t, *J* = 2.3 Hz, 1 H), 2.31 (dd, *J* = 2.2, 6.9 Hz, 2 H), 1.81 (dq, *J* = 4.0, 7.2 Hz, 1 H), 1.60 - 1.40 (m, 4 H), 1.40 - 1.01 (m, 8 H), 0.82 - 0.62 (m, 6 H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 203.0, 51.0, 34.4, 34.1, 32.8, 32.5, 28.1, 23.8, 7.3; IR (neat), ν: 2963, 2917, 2878, 2854, 2711, 1724, 1459, 1379, 1152, 1008, 905, 759; GC retention time: 10.69 min (method: program 2); MS (EI): 182 (M^+), 153 $(M\text{-}CH_2CH_3)^+$.

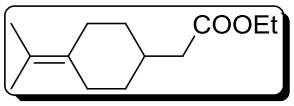


Ethyl 2-(4-oxocyclohexylidene)acetate (2.25)[13]: 60% NaH in mineral oil (1.32 g, 33 mmol) was washed with pentane and suspended in dry THF (150 mL) in ice bath. Triethylphosphonoacetate (7.8 g, 34.8 mmol) in THF (60 mL) was added dropwise. The mixture was stirred for 30 min, and added dropwise to cyclohexa-1,4-dione (3 g, 26.8 mmol) in THF (60 mL) at -30 °C. The mixture was stirred at -30 °C for 2h. It was then diluted with H_2O (150 mL) and extracted with EtOAc. The combined organic layers were washed with brine, dried and concentrated. The crude product was chromatographed with 20% EtOAc, 80% hexane to give 2.74 g (56%) colorless solid **2.25**. m. p.: 55-57 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.81 (s, 1H), 4.14 (q, *J* = 7.1 Hz, 2H), 3.17 (t, *J* = 6.5 Hz, 2H), 2.62 (t, *J* = 6.8 Hz, 2H), 2.45 (q, *J* = 6.2 Hz, 4H), 1.25 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 210.0, 166.0, 156.7, 116.0, 59.7, 39.4, 38.9, 33.7, 26.6, 14.2; IR (neat), ν: 2984, 2964, 2906, 1702, 1644, 1476, 1444, 1418, 1397, 1381, 1345, 1293, 1258, 1206, 1192, 1164, 1137, 1073,

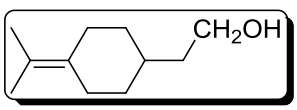
1036, 983, 941, 927, 874, 862, 799, 764, 750, 737; GC retention time: 13.39 min (method: program 4); MS (EI): 182 (M^+).



Ethyl 2-(4-oxocyclohexyl)acetate (2.26)[17]: Hydrogenation of ethyl 2-(4-oxycyclohexylidene)acetate (2.7 g, 14.8 mmol) as the general procedure described above to give colorless liquid **2.26**. Yield: 99%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.13 (q, *J* = 7.1 Hz, 2H), 2.41–2.25 (m, 6H), 2.12–1.98 (m, 3H), 1.45 (dt, *J* = 21.1, 9.9 Hz, 2H), 1.25 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 211.1, 172.3, 60.3, 40.4, 40.1, 32.3, 14.1; IR (neat), ν: 2934, 2860, 1727, 1712, 1448, 1418, 1369, 1344, 1279, 1246, 1201, 1151, 1114, 1095, 1029, 969, 754; GC retention time: 10.89 min (method: program 2); MS (EI): 184 (M^+).

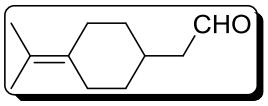


Ethyl 2-(4-(propan-2-ylidene)cyclohexyl)acetate (2.27): To an ice-cooled suspension of isopropyltriphenylphosphonium bromide (7.69g, 20 mmol) in dry THF (45 mL) was added 1.6 M *n*-BuLi (11.5 mL, 18.5 mmol) in hexanes. The mixture was stirred at 0 °C for 1 h. Ethyl 2-(4-oxocyclohexyl)acetate **2.26** (3.40g, 18.5 mmol) was added. The mixture was stirred at room temperature overnight. H_2O was added. The aqueous layer was extracted with ether. The combined organic layers were washed with brine, dried and concentrated. The crude product was chromatographed with 5% ether, 95% hexane to give colorless liquid **2.27**. Yield: 0.32g (10%). ^{1}H NMR (300MHz ,CHLOROFORM-d) δ = 4.14 (q, *J* = 7.0 Hz, 2 H), 2.64 (d, *J* = 14.4 Hz, 2 H), 2.19 (d, *J* = 7.0 Hz, 2 H), 2.12 - 1.86 (m, 2 H), 1.86 - 1.61 (m, 10 H), 1.27 (t, *J* = 7.0 Hz, 3 H), 1.10 - 0.86 (m, 3 H); GC retention time: 11.55 min (method: program 2); MS (EI): 210 (M^+).

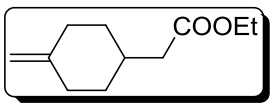


2-(4-(propan-2-ylidene)cyclohexyl)ethanol (2.28): LAH reduction of ethyl 2-(4-(propan-2-ylidene)cyclohexyl)acetate **2.27** (0.55

g, 2.62 mmol) as the general procedure described above to give colorless liquid **2.28**. Yield: 80%. ^{1}H NMR (300MHz, $CDCl_3$) δ 3.71 (t, *J* = 6.7 Hz, 2 H), 2.72 - 2.53 (m, 2 H), 1.94 - 1.70 (m, 4 H), 1.70 - 1.43 (m, 9 H), 1.32 - 1.15 (m, 1 H), 1.09 - 0.83 (m, 2 H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 131.5, 120.4, 60.8, 39.7, 34.3, 34.1, 29.4, 19.9; IR (neat), ν: 3321, 2961, 2913, 2845, 1444, 1373, 1053, 1018, 966; GC retention time: 10.83 min (method: program 2); MS (EI): 168 (M^+).

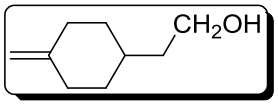


2-(4-(propan-2-ylidene)cyclohexyl)acetaldehyde (2.9): PCC oxidation of 2-(4-propan-2-ylidene)cyclohexyl)ethanol **2.28 (**0.2 g, 1.19 mmol) as the general procedure described above to give colorless liquid **2.9**. Yield: 60%. ^{1}H NMR (300MHz ,$CDCl_3$) δ 9.77 (t, *J* = 2.2 Hz, 1 H), 2.74 - 2.55 (m, 2 H), 2.31 (dd, *J* = 2.3, 6.7 Hz, 2 H), 2.05 (ddd, *J* = 3.7, 7.4, 14.4 Hz, 1 H), 1.89 - 1.69 (m, 4 H), 1.69 - 1.55 (m, 6 H), 1.15 - 0.78 (m, 2 H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 202.8, 130.4, 121.1, 50.7, 34.0, 32.7, 29.3, 19.9; IR (neat), ν: 2964, 2913, 2848, 2712, 1723, 1444, 1373, 1261, 1182, 1127, 1073, 1023, 899; GC retention time: 10.30 min (method: program 2); MS (EI): 166 (M^+).

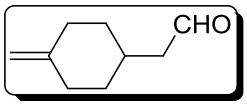


Ethyl 2-(4-methylenecyclohexyl)acetate (2.32)[18]: Prepared from Ethyl 2-(4-oxocyclohexyl)acetate **2.26** (3 g, 16.3 mmol) and methyltriphenylphosphonium bromide (7 g, 19.5 mmol) by the same procedure as **2.27** to give colorless liquid **2.32**. Yield: 74%. ^{1}H NMR (300MHz ,$CDCl_3$) δ 4.60 (s, 2 H), 4.12 (dq, *J* = 0.9, 7.1 Hz, 2 H), 2.28 (d, *J* = 13.8 Hz, 2 H), 2.19 (d, *J* = 7.0 Hz, 2 H), 2.05 (dt, *J* = 4.1, 13.3 Hz, 2 H), 1.94 (ddd, *J* = 3.8, 7.4, 11.1 Hz, 1 H), 1.88 - 1.77 (m, 2 H), 1.25 (dt, *J* = 0.7, 7.1 Hz, 3 H), 1.07 (dq, *J* = 3.7, 12.3 Hz, 2 H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 172.9, 148.8, 107.1, 60.1, 41.3, 34.3, 34.0, 33.9, 14.4, 14.1; IR (neat), ν: 3071, 2981, 2932, 2850, 1733, 1651, 1444, 1370, 1342, 1276,

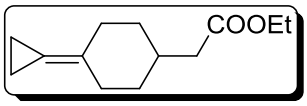
1244, 1206, 1147, 1085, 1031, 886; GC retention time: 11.81 min (method: program 4); MS (EI): 182 (M^+)



2-(4-methylenecyclohexyl)ethanol: LAH reduction of ethyl 2-(4-methylenecyclohexyl)acetate **2.32** (2 g, 11.0 mmol) as the general procedure described above to give colorless liquid. Yield: 97%. ^{1}H NMR (300MHz ,$CDCl_3$) δ 4.59 (s, 2 H), 3.67 (t, *J* = 6.6 Hz, 2 H), 2.28 (d, *J* = 13.5 Hz, 2 H), 2.12 - 1.93 (m, 2 H), 1.90 - 1.73 (m, 3 H), 1.65 - 1.40 (m, 3 H), 1.16 - 0.92 (m, 2 H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 149.6, 106.7, 60.7, 39.4, 34.5, 34.3, 33.7; IR (neat), ν: 3321, 3070, 2923, 2849, 1650, 1446, 1086, 1054, 1024, 964, 885; GC retention time: 9.17 min (method: program 2); MS (EI): 140 (M^+).

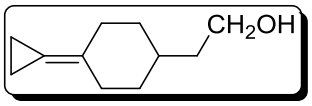


2-(4-methylenecyclohexyl)acetaldehyde (2.12): Prepared by PCC oxidation of 2-(4-methylenecyclohexyl)ethanol **2.32** (0.3 g, 2.14 mmol) as the general procedure described above to give colorless liquid **2.12**. Yield: 60%. ^{1}H NMR (300MHz ,$CDCl_3$) δ 9.76 (t, *J* = 2.1 Hz, 1 H), 4.61 (s, 2 H), 2.42 - 2.22 (m, 4 H), 2.16 - 1.96 (m, 3 H), 1.90 - 1.76 (m, 2 H), 1.10 (dq, *J* = 4.0, 12.3 Hz, 2 H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 202.3, 148.4, 107.3, 50.5, 34.2, 34.1, 32.0; IR (neat), ν: 3071, 2919, 2850, 2716, 1722, 1650, 1446, 1406, 1195, 1175, 1100, 1029, 1003, 887, 645; GC retention time: 8.39 min (method: program 2); MS (EI): 138 (M^+).

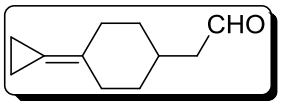


Ethyl 2-(4-cyclopropylidenecyclohexyl)acetate (2.29): Prepared by the same procedure as **2.27**. The crude product was chromatographed with 5% ether, 95% hexane to give colorless liquid **2.29**. Yield: 40%. ^{1}H NMR (300MHz ,$CDCl_3$) δ 4.12 (q, *J* = 7.0 Hz, 2 H), 2.56 - 2.40 (m, 2 H), 2.20 (d, *J* = 7.3 Hz, 2 H), 2.16 - 1.87 (m, 3 H), 1.87 - 1.76 (m, 2 H), 1.25 (t, *J* = 7.2 Hz, 3 H), 1.15 - 0.89 (m, 6 H); ^{13}C

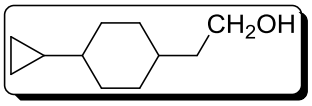
NMR (75MHz ,$CDCl_3$) δ 173.0, 126.8, 112.4, 60.1, 41.5, 34.8, 33.6, 32.4, 14.3, 1.7; IR (neat), ν: 2977, 2933, 2848, 1732, 1442, 1368, 1341, 1282, 1239, 1156, 1110, 1031, 965, 931, 854; GC retention time: 11.70 min (method: program 2); MS (EI): 208 (M^+).



2-(4-cyclopropylidenecyclohexyl)ethanol (2.30): Prepared by LAH reduction of **2.29** (1.3 g, 6.24 mmol) as the general procedure described above to give colorless liquid **2.30**. Yield: 92%. ^{1}H NMR (300MHz ,$CDCl_3$) δ 3.70 (t, *J* = 6.6 Hz, 2 H), 2.55 - 2.45 (m, 2 H), 2.14 - 1.98 (m, 2 H), 1.94 - 1.78 (m, 2 H), 1.69 - 1.46 (m, 4 H), 1.25 - 0.92 (m, 6 H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 127.6, 112.0, 60.8, 39.6, 34.2, 34.0, 32.7, 1.7; IR (neat), ν: 3319, 3044, 2974, 2915, 2847, 1440, 1412, 1260, 1193, 1126, 1086, 1053, 1019, 991, 958, 915, 867, 815, 756, 690; GC retention time: 11.03 min (method: program 2); MS (EI): 166 (M^+).

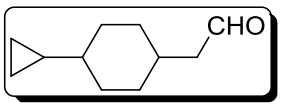


2-(4-cyclopropylidenecyclohexyl)acetaldehyde (2.10): Prepared by PCC oxidation of **2.30** (0.3 g, 1.80 mmol) as the general procedure described above. The crude product was purified by column chromatography with 5% ether and 95% hexane to give colorless liquid **2.10**. Yield: 60%. ^{1}H NMR (300MHz ,$CDCl_3$) δ 9.78 (t, *J* = 2.2 Hz, 1 H), 2.50 (d, *J* = 13.8 Hz, 2 H), 2.34 (dd, *J* = 2.1, 6.7 Hz, 2 H), 2.23 - 1.98 (m, 3 H), 1.93 - 1.76 (m, 2 H), 1.23 - 0.86 (m, 6 H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 202.6, 126.5, 112.8, 50.7, 33.8, 32.5, 1.7; IR (neat), ν: 3044, 2975, 2915, 2834, 2714, 1722, 1441, 1412, 1348, 1275, 1191, 1176, 1131, 1069, 1000, 916, 897, 757, 691; GC retention time: 10.47 min (method: program 2); MS (EI): 164 (M^+).

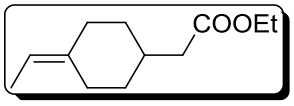


2-(4-cyclopropylcyclohexyl)ethanol (2.31): To a solution of 0.4 g 2-(4-cyclopropylidenecyclohexyl)ethanol (**2.30**) in 20 mL CH_2Cl_2

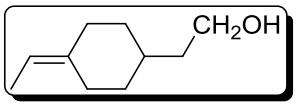
was added o-nitrobenzenesulfonylhydrazide (NBSH) (1.68 g, 7.73 mmol, prepared according to the procedure reported by Myer and coworkers[8]) followed by triethylamine (2.15 mL, 15.4 mmol). The mixture was stirred for 15 h and then washed with sat. $NaHCO_3$ solution. The organic layers were dried with $MgSO_4$ and evaporated. The crude product was purified by column chromatography (30% ether and 70% hexane) to give 0.3 g (74%) pale yellow liquid **2.31**. ^{1}H NMR (300MHz ,$CDCl_3$) δ 3.66 (dt, *J* = 1.9, 6.7 Hz, 2 H), 1.90 - 1.66 (m, 3 H), 1.64 - 1.24 (m, 7 H), 1.15 - 0.96 (m, 1 H), 0.96 - 0.81 (m, 1 H), 0.81 - 0.66 (m, 1 H), 0.52 - 0.26 (m, 3 H), 0.10 - -0.06 (m, 2 H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 61.1, 60.8, 43.1, 41.2, 40.2, 37.6, 34.3, 33.2, 32.7, 32.1, 29.2, 29.1, 17.5, 14.8, 3.8, 3.0; IR (neat), ν: 3316, 3073, 2998, 2915, 2848, 1447, 1047, 1012, 889, 875, 816; GC retention time: 10.73 min and 10.81 min (mixture of ~1:1 *cis* and *trans* isomer) (method program 2); MS (EI): 150 $(M-H_2O)^+$.



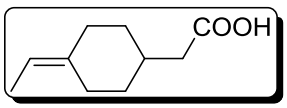
2-(4-cyclopropylcyclohexyl)acetaldehyde (2.11): Prepared by PCC oxidation of **2.31** (0.15 g, 0.89 mmol) as the general procedure described above. The crude product was purified by chromatography with 5% ether and 95% hexane to give colorless liquid **2.11**. Yield: 60%. ^{1}H NMR (300MHz ,$CDCl_3$) δ 9.75 (td, *J* = 2.3, 3.5 Hz, 1 H), 2.39 (dd, *J* = 2.3, 7.0 Hz, 1 H), 2.27 (dd, *J* = 2.2, 6.6 Hz, 1 H), 2.11 (td, *J* = 3.5, 7.0 Hz, 1 H), 1.99 - 1.66 (m, 3 H), 1.65 - 1.31 (m, 4 H), 1.23 - 1.03 (m, 1 H), 1.03 - 0.81 (m, 1 H), 0.54 - 0.26 (m, 3 H), 0.13 - -0.09 (m, 2 H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 203.0, 202.9, 51.3, 48.9, 42.7, 40.8, 33.1, 32.7, 32.5, 30.5, 29.2, 28.9, 17.4, 14.8, 3.8, 3.0; IR (neat), ν: 3073, 2998, 2918, 2850, 2711, 1723, 1448, 1396, 1290, 1164, 1109, 1015, 949, 891, 817; GC retention time: 10.20 min and 10.28 min (mixture of ~1:1 *cis* and *trans* isomer) (method: program 2); MS (EI): 166 (M^+).



Ethyl 2-(4-ethylidenecyclohexyl)acetate (2.33): Prepared from Ethyl 2-(4-oxocyclohexyl)acetate **2.26** (1 g, 5.43 mmol) and ethyltriphenylphosphonium bromide (2.42 g, 6.51 mmol) by the same procedure as **2.27** to give 0.8 g (75%) colorless liquid **2.33** (contains small amount of *n*-butyl ester). ^{1}H NMR (300MHz ,$CDCl_3$) δ 5.15 (q, *J* = 6.7 Hz, 1 H), 4.12 (q, *J* = 7.2 Hz, 2 H), 2.65 - 2.54 (m, 1 H), 2.24 - 2.12 (m, 3 H), 2.11 - 1.88 (m, 2 H), 1.86 - 1.67 (m, 3 H), 1.64 - 1.52 (m, 3 H), 1.33 - 1.20 (m, 3 H), 1.11 - 0.89 (m, 2 H); GC retention time: 7.98, 9.18(small amount of *n*-butylester) (method: program 3) min; MS: 196 (M^+), 224 (M^+)

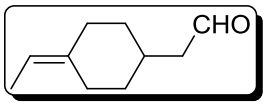


2-(4-ethylidenecyclohexyl)ethan-1-ol (2.34): **P**repared by LAH reduction of **2.33** (0.74 g, 4.4 mmol) as the general procedure described above to give colorless liquid **2.34**. Yield: 97%. ^{1}H NMR (300MHz ,$CDCl_3$) δ 5.16 (q, *J* = 6.7 Hz, 1 H), 3.75 - 3.65 (m, 2 H), 2.69 - 2.51 (m, 1 H), 2.23 - 2.13 (m, 1 H), 2.10 - 1.95 (m, 1 H), 1.88 - 1.65 (m, 4 H), 1.65 - 1.44 (m, 6 H), 1.36 (s, 1 H), 1.09 - 0.87 (m, 2 H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 139.5, 115.3, 60.8, 39.6, 36.1, 34.6, 34.3, 33.7, 27.3, 12.6; IR (neat), ν: 3340 (br), 2921, 2852, 1444, 1381, 1087, 1053, 1018, 818; GC retention time: 10.13 min (method program 2); MS (EI): 154 (M^+)

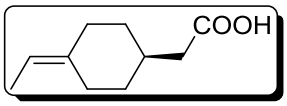


2-(4-ethylidenecyclohexyl)acetic acid (2.35): **2.33** (6 g, 30.6 mmol) was refluxed with 2N KOH solution (30 mL) in 50% methanol for 1 h. After cooling to room temperature, the mixture was diluted with 25 mL water and extracted with ether three times. The aqueous layer was acidified with HCl and extracted with ether. The combined ether layers were dried and evaporated to give colorless crystal **2.35** (3.9 g, 75%). m.p.: 60-62 °C; ^{1}H NMR (300MHz ,$CDCl_3$) δ 5.17 (q, *J* = 6.6 Hz, 1 H), 2.69 - 2.55 (m, 1 H), 2.27 (d, *J* = 6.7 Hz, 2 H), 2.24 - 2.14 (m, 1 H), 2.12 - 1.75 (m, 5 H), 1.57 (td, *J* = 1.4, 6.5 Hz,

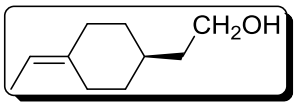
3 H), 1.17 - 0.93 (m, 2 H); IR (neat), ν: 2964, 2923, 2841, 1696, 1405, 1292, 1164, 936, 820; GC retention time: 7.77 min (method program 3); MS (EI): 168 (M^+)



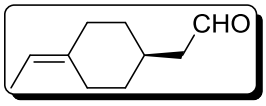
2-(4-ethylidenecyclohexyl)acetaldehyde (2.6): Prepared by PCC oxidation of **2.34** (0.3 g, 0.89 mmol) as the general procedure described above. The crude product was purified by chromatography with 5% ether and 95% hexane to give colorless liquid **2.6**. Yield: 60%. ^{1}H NMR (300MHz ,$CDCl_3$) δ 9.75 (t, *J* = 2.1 Hz, 1 H), 5.15 (q, *J* = 6.4 Hz, 1 H), 2.68 - 2.51 (m, 1 H), 2.31 (dd, *J* = 2.1, 6.7 Hz, 2 H), 2.23 - 2.12 (m, 1 H), 2.12 - 1.96 (m, 2 H), 1.88 - 1.68 (m, 3 H), 1.55 (quind, *J* = 1.5, 3.8 Hz, 3 H), 1.15 - 0.91 (m, 2 H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 202.5, 138.4, 116.0, 50.7, 35.9, 34.5, 33.5, 32.7, 27.1, 12.6; IR (neat), ν: 2918, 2850, 2715, 1722, 1443, 1383, 1174, 1106, 1021, 897, 818; GC retention time: 13.18 min (method: program 5); MS (EI): 152 (M^+)



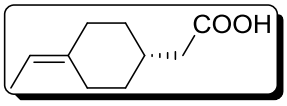
(R,Z)-2-(4-ethylidenecyclohexyl)acetic acid (2.37): Chiral resolution of rac-2-(4-ethylidenecyclohexyl)acetic acid: rac-2-(4-ethylidenecyclohexyl)acetic acid (**2.35**) (1.7 g, 0.01 mol) and (+)-methylbenzylamine (1.22 g, 0.01 mol) was dissolved in 12 mL EtOAc. The solution was heated to 60 °C and cooled to room temperature. Crystal slowly formed after 1h. The solid was filtered and recrystallized from EtOAc again to give 1.3 g of ammonium salt. The ammonium salt was stirred with 1N NaOH for 1 h. The mixture was extracted with ether. The aqueous layer was acidified with HCl and extracted with ether. The combined organic layers were washed with brine, dried and evaporated to give 0.65 g colorless solid. ^{1}H NMR (300MHz ,$CDCl_3$) δ 5.17 (q, *J* = 6.6 Hz, 1 H), 2.69 - 2.55 (m, 1 H), 2.27 (d, *J* = 6.7 Hz, 2 H), 2.24 - 2.14 (m, 1 H), 2.12 - 1.75 (m, 5 H), 1.57 (td, *J* = 1.4, 6.5 Hz, 3 H), 1.17 - 0.93 (m, 2 H); IR (neat), ν: 2964, 2923, 2841, 1696, 1405, 1292, 1164, 936, 820; GC retention time: 7.77 min (method: program 3); MS (EI): 168 (M^+)



(R,Z)-2-(4-ethylidenecyclohexyl)ethan-1-ol (2.39): **P**repared by LAH reduction of **2.37** (1 g, 5.94 mmol) as the general procedure described above to give colorless liquid **2.39**. Yield: 97%. ^{1}H NMR (300MHz ,$CDCl_3$) δ 5.16 (q, *J* = 6.7 Hz, 1 H), 3.75 - 3.65 (m, 2 H), 2.69 - 2.51 (m, 1 H), 2.23 - 2.13 (m, 1 H), 2.10 - 1.95 (m, 1 H), 1.88 - 1.65 (m, 4 H), 1.65 - 1.44 (m, 6 H), 1.36 (s, 1 H), 1.09 - 0.87 (m, 2 H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 139.5, 115.3, 60.8, 39.6, 36.1, 34.6, 34.3, 33.7, 27.3, 12.6; IR (neat), ν: 3340 (br), 2921, 2852, 1444, 1381, 1087, 1053, 1018, 818; GC retention time: 10.13 min (method: program 2); MS (EI): 154 (M^+)

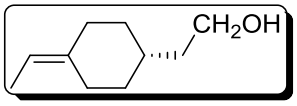


(R,Z)-2-(4-ethylidenecyclohexyl)acetaldehyde (2.8): Prepared by PCC oxidation of **2.39** (0.3 g, 0.89 mmol) as the general procedure described above. The crude product was purified by chromatography with 5% ether and 95% hexane to give colorless liquid **2.6**. Yield: 60%. ^{1}H NMR (300MHz ,$CDCl_3$) δ 9.75 (t, *J* = 2.1 Hz, 1 H), 5.15 (q, *J* = 6.4 Hz, 1 H), 2.68 - 2.51 (m, 1 H), 2.31 (dd, *J* = 2.1, 6.7 Hz, 2 H), 2.23 - 2.12 (m, 1 H), 2.12 - 1.96 (m, 2 H), 1.88 - 1.68 (m, 3 H), 1.55 (quind, *J* = 1.5, 3.8 Hz, 3 H), 1.15 - 0.91 (m, 2 H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 202.5, 138.4, 116.0, 50.7, 35.9, 34.5, 33.5, 32.7, 27.1, 12.6; IR (neat), ν: 2918, 2850, 2715, 1722, 1443, 1383, 1174, 1106, 1021, 897, 818; GC retention time: 13.18 min (method: program 5); MS (EI): 152 (M^+).

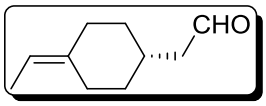


(S,E)-2-(4-ethylidenecyclohexyl)acetic acid (2.36): Prepared by the same procedure (with (-)-methylbenzylamine) as **2.37.** ^{1}H NMR (300MHz ,$CDCl_3$) δ 5.17 (q, *J* = 6.6 Hz, 1 H), 2.69 - 2.55 (m, 1 H), 2.27 (d, *J* = 6.7 Hz, 2 H), 2.24 - 2.14 (m, 1 H), 2.12 - 1.75 (m, 5 H), 1.57 (td, *J* = 1.4, 6.5 Hz, 3 H), 1.17 - 0.93 (m, 2 H); IR

(neat), ν: 2964, 2923, 2841, 1696, 1405, 1292, 1164, 936, 820; GC retention time: 7.77 min (method: program 3); MS (EI): 168 (M^+)



(S,E)-2-(4-ethylidenecyclohexyl)ethan-1-ol (2.38): Prepared by LAH reduction of **2.36** (1 g, 5.94 mmol) as the general procedure described above to give colorless liquid **2.38**. Yield: 97%. ^{1}H NMR (300MHz ,$CDCl_3$) δ 5.16 (q, *J* = 6.7 Hz, 1 H), 3.75 - 3.65 (m, 2 H), 2.69 - 2.51 (m, 1 H), 2.23 - 2.13 (m, 1 H), 2.10 - 1.95 (m, 1 H), 1.88 - 1.65 (m, 4 H), 1.65 - 1.44 (m, 6 H), 1.36 (s, 1 H), 1.09 - 0.87 (m, 2 H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 139.5, 115.3, 60.8, 39.6, 36.1, 34.6, 34.3, 33.7, 27.3, 12.6; IR (neat), ν: 3336 (br), 2921, 2852, 1444, 1381, 1087, 1053, 1018, 818; GC retention time: 10.13 min (method: program 2); MS (EI): 154 (M^+)



(S,E)-2-(4-ethylidenecyclohexyl)acetaldehyde (2.7): Prepared by PCC oxidation of **2.38** (0.3 g, 0.89 mmol) as the general procedure described above. The crude product was purified by chromatography with 5% ether and 95% hexane to give colorless liquid **2.6**. Yield: 60%. ^{1}H NMR (300MHz ,$CDCl_3$) δ 9.75 (t, *J* = 2.1 Hz, 1 H), 5.15 (q, *J* = 6.4 Hz, 1 H), 2.68 - 2.51 (m, 1 H), 2.31 (dd, *J* = 2.1, 6.7 Hz, 2 H), 2.23 - 2.12 (m, 1 H), 2.12 - 1.96 (m, 2 H), 1.88 - 1.68 (m, 3 H), 1.55 (quind, *J* = 1.5, 3.8 Hz, 3 H), 1.15 - 0.91 (m, 2 H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 202.5, 138.4, 116.0, 50.7, 35.9, 34.5, 33.5, 32.7, 27.1, 12.6; IR (neat), ν: 2918, 2850, 2715, 1722, 1443, 1383, 1174, 1106, 1021, 897, 818; GC retention time: 13.18 min (method: program 5); MS (EI): 152 (M^+).

Chapter 3: Equilibrium-upset analogs reveal unexpected GPCR activation chemistry at aldehyde odorant receptors

3.1 Introduction

The aldehyde functional group is common among natural product odorants.[1] Aldehydes are widely used in perfumery, such as heptanal (fruity odor), octanal (fruity odor), citral (lemon odor), etc. Lots of odorants contain a polar heteroatom containing functional group that forms stronger polar intermolecular interaction with ORs, and a hydrophobic carbon chain that likely forms weaker hydrophobic interaction with ORs. The polar functional group plays a critical role in determining the smell of the molecule. The aldehydes synthesized in Chapter 1 and Chapter 2 all have a characteristic "aldehydic" smell similar to octanal and heptanal, while their corresponding alcohol precursor all have a green smell similar to octanol. In this chapter, we study how the olfactory system recognizes the aldehyde functional group and may distinguishes it from related functional groups.

To study the recognition of aldehyde by ORs, some theoretical calculations have been done on aldehyde ORs based on the structure of rhodopsin[2]. These studies usually only consider the carbonyl form of the aldehyde. However, when an aldehyde passes through the aqueous based layer of nasal mucus to reach the ORs, up to ~45% of it (estimated from the hydration constant K_{hyd}= ~0.83[3]) could be converted to its gem-diol hydrate (Figure 3.1). Comparing to the carbonyl form of aldehyde, which contains only one oxygen atom as hydrogen bond acceptor, the gem-diol form contains two oxygen atoms as hydrogen bond acceptors and two hydrogen as hydrogen bond donors. The gem-diol form can potentially form stronger H-bond interactions with ORs. Moreover, the gem-diol form has different shape (sp^3 vs sp^2) and size (extra size from the second

–OH group) than the carbonyl form. To effectively interact with the gem-diol form, ORs will need to use different receptor binding site strategies than those that specifically bind the carbonyl form. Therefore, it's worth studying the possibility that some ORs that are activated by octanal actually are activated by the gem-diol form.

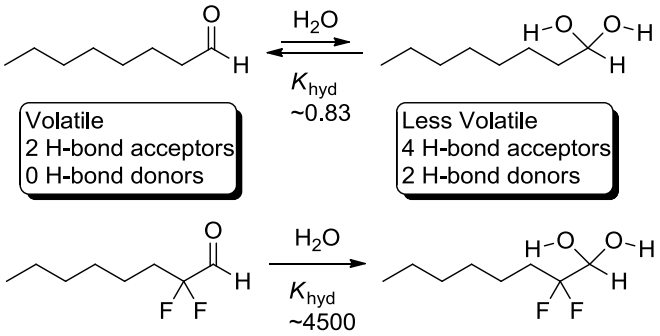


Figure 3.1 Aldehyde hydration equilibria and H-bonding

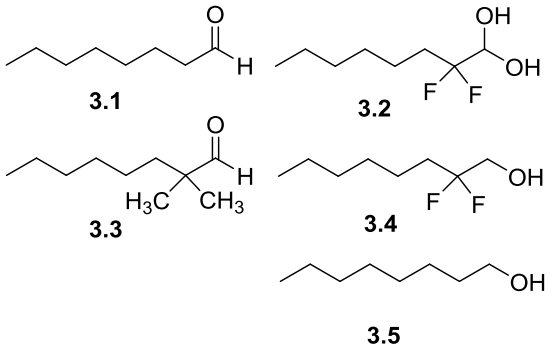


Figure 3.2 Octanal and analogs used in panel screen

Four compounds were chosen to be tested and compared to octanal. 2,2-difluorooctanal (**3.2,** Figure 3.5) was used as a gem-diol hydrate analogue of octanal. The two electron-withdrawing fluorine groups at C_2 shift the hydration equilibrium to favor the gem-diol form. Estimated from the K_{hyd} of 2,2-difluorononanal (~4500)[4], **3.2** exists almost completely in its gem-diol form in water. In contrast, the two electron donating methyl groups of 2,2-dimethyloctanal (**3.3,** Figure 3.5) shift the hydration equilibrium to favor the carbonyl form. A side effect of introducing

substitution at C_2 is that it also introduces some steric bulkiness at C_2. As the Van der Waal radius increase from H (1.20 Å) to F (1.47 Å) and $–CH_3$(2.0 Å), the corresponding compound may show lower potency to some ORs that are sensitive to the steric effect. The comparison between 2,2-difluorooctanol (**3.4**) and octanol (**3.5**) will help us to closely monitor the steric effect of fluorine. Also, the comparison between gem-diol **3.2** and alcohol **3.4** will provide us with details of how the second hydroxyl group on hydrate affects the recognition.

OR-I7 has previously shown to be activated specifically by aldehyde but not by other functional groups. ORs like OR-I7 are very important in determine the aldehydic smell of octanal. In this chapter, instead of looking at one specific receptor (OR-I7), the five compounds (**3.1**-**3.5**) are tested by panel screen with large amount of different ORs (by our collaborators Zita Peterlin and Stuart Firestein at Columbia University). First, over 1000 rat olfactory sensory neurons (ORNs) are tested by octanal and the cells that have response from octanal are selected. Then these cells are tested by **3.2**, **3.3**, **3.4** and **3.5**. We are trying to understand how the olfactory system recognizes aldehydes: what percentage of ORs specifically recognize aldehyde group; and how many recognize the gem-diol hydrate form?

3.2 Synthesis and Equilibrium Study of 2,2-Difluorooctanal and 2,2-Dimethyloctanal

Synthesis of 2,2-difluorooctanal and 2,2-dimethyloctanal

Two methods of synthesizing 2,2-difluoroaldehyde have been reported. Bernard and Gobind reported synthesis of 2,2-difluoroaldehyde by DIBAL-H reduction of 2,2-difluoroacid ester[5]. Carmen and coworker studied the oxidation of 2,2-difluoroalcohol to 2,2-difluoroaldehyde and reported that the best condition was Swern oxidation with moderate yield (35-66%)[4]. From previous experience, aldehyde prepared by Swern oxidation has a foul odor of side product

dimethylsulfide which can be detected by human nose in concentration as low as parts per billion. In order to avoid possible interference of dimethylsulfide, the Bernhard and Gobind's method was chosen for synthesizing **3.2** (Figure 3.2).

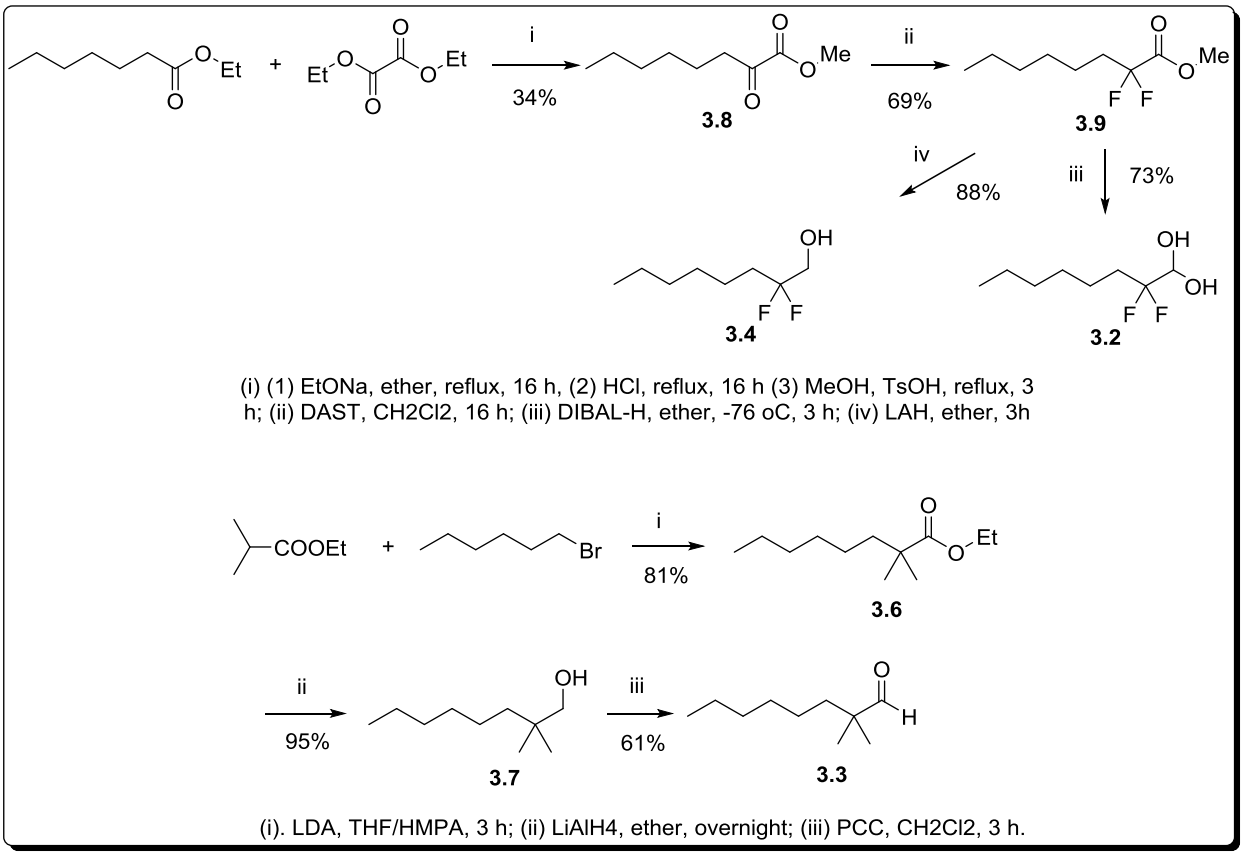


Figure 3.3 Synthesis of 2,2-difluorooctanal and 2,2-dimethyloctanal

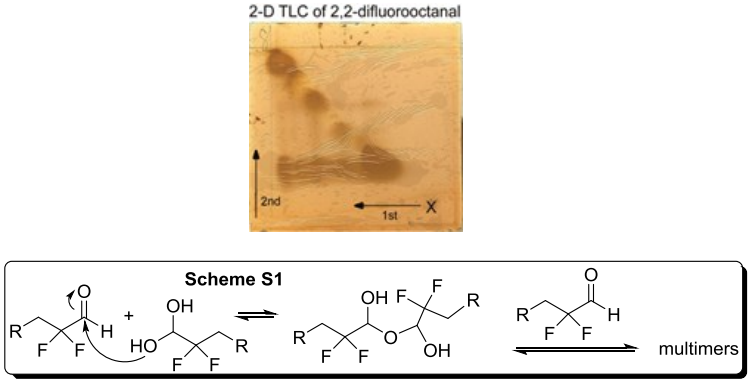


Figure 3.4 Equilibrium of 2,2-difluorooctanal to form multimeric hemiacetals

When analyzed by TLC with iodine visualization, the purified difluoroaldehyde **3.2** showed spots that appeared to interconvert on the plate. We interpreted this behavior as visualization of the silica-catalyzed equilibrium of monomer aldehyde forms with multimeric hemiacetals (Figure 3.3). Off-diagonal TLC spots on 2-dimensional TLC showed that all the forms can interconvert, during the dilution process of the TLC elution, to one major form, probably a monomer form (R_f ~0.25, 1:1 ether:hexanes). The ^{1}H-NMR of the freshly chromatographed product also showed peaks around 4-5 ppm which may be C-1 proton of the multimers. When dissolved in D_2O, however, the multimers appeared to hydrolyze rapidly to the aldehyde hydrate by ^{1}H-NMR (shown below), and thus the equilibrium did not affect the biological testing. Furthermore, by GC/MS, only one peak was observed for the purified product and the fragmentation pattern matched that of the dehydrated difluoroaldehyde. The high temperature of the injection port (280 ºC) likely breaks up the multimers and dehydrates the gem-diol to the carbonyl form of the difluoroaldehyde. The 2,4-DNP derivatives were made to furnish a single species for characterization.

Effect of 2,2-difluorosubstitution on aldehyde hydrates, imine and acetal equilibrium

Before testing the compounds, we studied the effect of 2,2-difluorosubstitution by ^{1}H NMR. Due to the low solubility of octanal and 2,2-difluorooctanal in water, the equilibrium of octanal and 2,2-difluorooctanal couldn't be directly measured by ^{1}H NMR. Since previous study showed that the number of carbons doesn't affect the hydration equilibrium (from *n*-propanal to *n*-hexanal)[3], we choose to use shorter aldehydes to estimate the equilibrium of octanal in water. Hexanal, the longest *n*-aldehyde that is still soluble enough in water was used as a replacement of octanal. Similarly, 2,2-dimethylhexanal (**3.3b**) was used as a replacement of 2,2-dimethyloctanal (**3.3**). 2,2-difluoroheptanal (**3.2b**) was used as a replacement of 2,2-

difluorooctanal (**3.2**) as it's the shortest 2,2-dlfuoroaldehyde that we were able to work with in the pure form (2,2-difluorohexanal was too volatile).

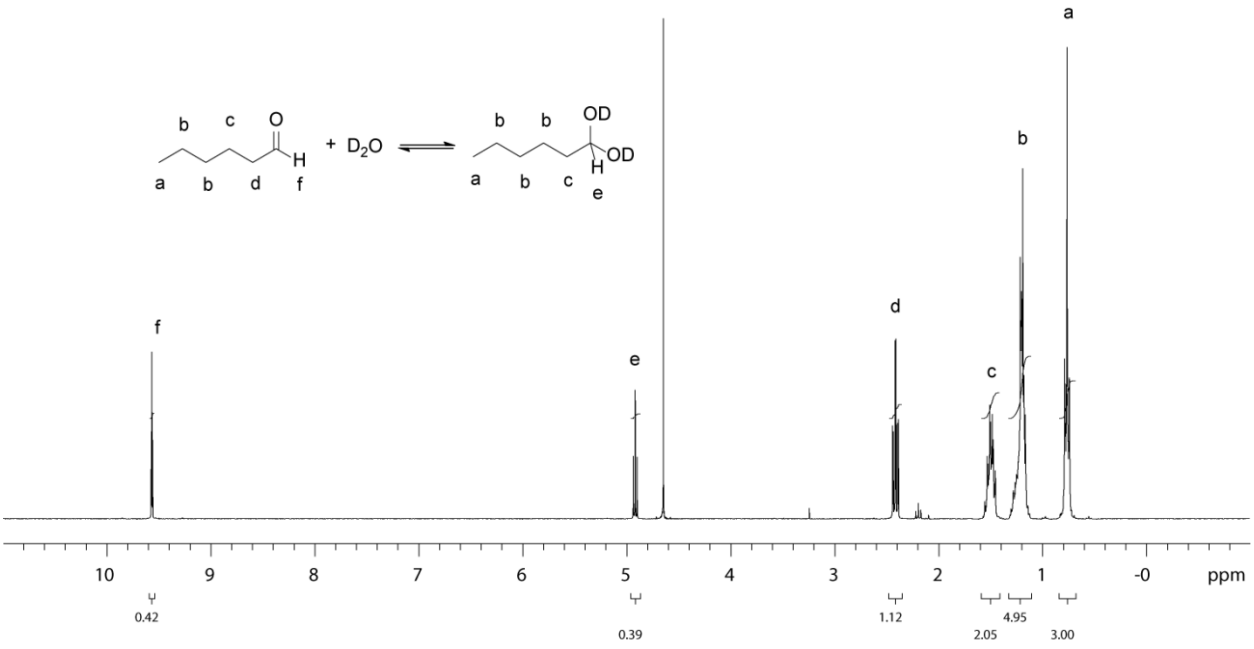


Figure 3.5 ^{1}HNMR of *n*-hexanal in D_2O

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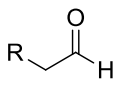
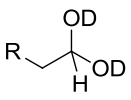
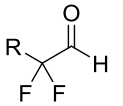
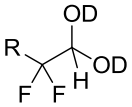
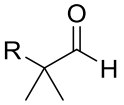
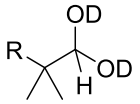
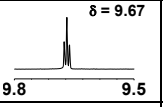
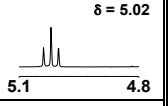
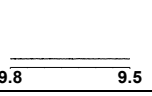
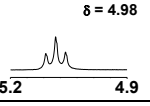
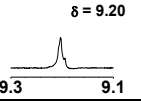
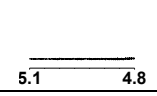
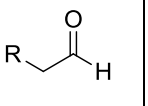
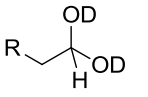
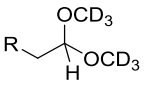
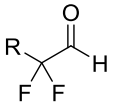
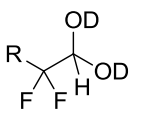
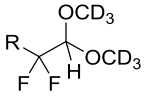
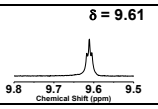
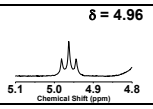
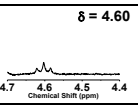
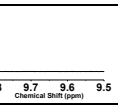
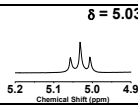
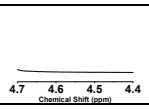
						
Spectrum						
Chemical shift	9.67 ppm (ALD)	5.02 ppm (HYD)	-	4.98 ppm (HYD)	9.20 ppm (ALD)	-
In D2O	57%	43%	0%	100%	100%	0%

Table 3.1 Hydration equilibrium of aldehydes by ^{1}HNMR

First, hexanal, 2,2-difluoroheptanal (**3.2b**) and 2,2-dimethylhexanal (**3.3**) were checked by ^{1}HNMR in D_2O. A typical ^{1}HNMR result of hexanal in D_2O is shown in Figure 3.4. The signal of aldehyde proton (-CHO) was observed at 9.67 ppm, and the signal of hydrate proton (-$CH(OD)_2$) was observed at 5.02 ppm. The hydration equilibrium constant was calculated based

on these two signals to be K_{hyd} = 0.75, which closely matched the reported K_{hyd} (0.83)[3b]. As expected, 2,2-difluoroheptanal showed only signals for its hydrate form without any detectable aldehyde signal. In contrast, 2,2-dimethylhexanal in D_2O showed only signals for the carbonyl form. Therefore, 2,2-difluoroaldehyde and 2,2-dimethylaldehyde provide two extreme examples of the two aldehyde forms: carbonyl form and gem-diol hydrate form.

	Aldehyde			Difluoroaldehyde		
Structure						
Spectrum						
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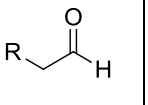
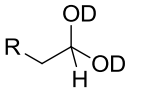
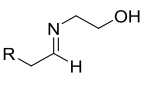
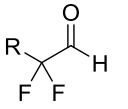
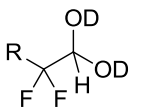
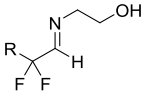
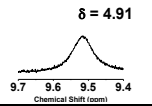
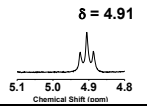
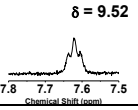
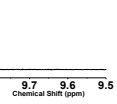
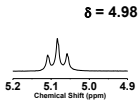
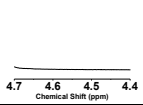
In D2O	47%	43%	10%	0%	100%	0%
	Aldehyde			Difluoroaldehyde		
Structure						
Spectrum						
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In D2O	43%	46%	11%	0%	100%	0%

Table 3.2 Effect of 2,2-difluoro substitution on formation of imine and acetal

In the OR binding site, -OH and $–NH_2$ groups from amino acid side chain can potentially form acetal and imine bond with aldehyde which increase the affinity between ORs and aldehydes. The difluoro substitution on the C_2 may change the reactivity of the aldehyde group with hydroxyl and amino group in a way that it increases the potency of aldehyde. We wanted to make sure that the increased potency of 2,2-difluorooctanal, if we observe any, are not due to the

increased reactivity of the aldehyde group towards the formation of acetal or imine but rather due to the increased hydration. When 30 equivalents of methanol-D_4 was mixed with hexanal, only 10% hexanal was converted the acetal. For 2,2-difluoroheptanal, no formation of acetal was observed, indicating that the fluorines do not make the aldehyde group more reactive with alcohols. The ^{1}H NMR signal at ~4.60 ppm should be acetal hydrogen rather than hemiacetal for following reasons: 1) acetals are normally considered more stable than hemiacetals, so if there is a peak for hemiacetals, we should also clearly see a peak for acetal; 2) the chemical shift of hydrate, hemiacetals, acetal should be in the order of hydrate>hemiacetals>acetal. Based on these two points, if the 4.6 ppm signal is hemiacetals, then we should see another signal slightly to the right of it representing acetal which was not seen on the NMR spectrum. So the 4.6 ppm peak is likely acetal. If it's true, what we don't know now is whether there is a peak for hemiacetal, because it's likely buried under the water peak. But it shouldn't be much if there is any, because the integration of aldehyde, hydrate, acetal already almost add up to 1.

Butylamine (0.3 equivalents) was first used to mix with hexanal and 2,2-difluoroheptanal (data not shown). The solution started to turn cloudy after 5-30 min (depends on the concentration) probably due to the low solubility of the imine product. To increase the solubility of the imine, ethanolamine (0.3 equivalents) was used as a surrogate for amino group of possible receptor lysine (Same equivalent of alcohol was shown not to form acetal with the aldehyde). ~11% hexanal formed the imine while difluoroheptanal didn't react, again ensuring that the difluoro substitution reduces the reactivity toward amines. These results likely result from the fact that, in water, the electrophile carbonyl form of the aldehyde is absent. In summary, the 2,2-difluoro substitution increases the formation of gem-diol hydrate, but does not promote the formation of acetal or imine that may result in increased activation of ORs. We can be sure that

any differences in the biological results are due to hydration changes, not reactivity with nucleophiles in the receptor interior.

3.3 Results

The compounds were tested by Zita Peterlin in Prof. Stuart Firestein's lab at Columbia University. Different olfactory sensory neurons (over 1000) were isolated from the entire olfactory epithelium. The cells were loaded with a fluorescent Ca^{2+} indicator fura-2AM followed by application of the testing compounds. When a cell is activated by a compound, Ca^{2+} channels on the cell membrane will open. The increased Ca^{2+} that enters the cells during the signal transduction process forms a complex with fura-2, inducing a change of fluorescence which is recorded by a ccd camera and imaging software[7].

Out of 1053 cells observed, 87 cells (8%, Fig. 3.6, c1-c87) were activated by octanal. A summary of octanal-activated rat OSN responses to compounds **2.1**-**2.5** is shown in **Figure 3.6**. Responses for each compound are reported relative to the octanal response generated by that cell, which is set to 100% (red in color scale). The cells are arranged in groups with different response pattern for the 5 compounds tested.

In terms of molecular shape and size, octanol is one of the closest analogues of octanal. Octanal and octanol share many structure aspects without extra bulkiness. This similarity between octanal and octanol is reflected on the result. Out of 87 cells that were activated by octanal, 59 cells (68%, c13-c26, c41-c70, c73-c87, Fig. 3.6) were also activated by octanol. These cells cannot distinguish octanal from octanol, and are likely not aldehyde specific receptors (Figure 3.7a).

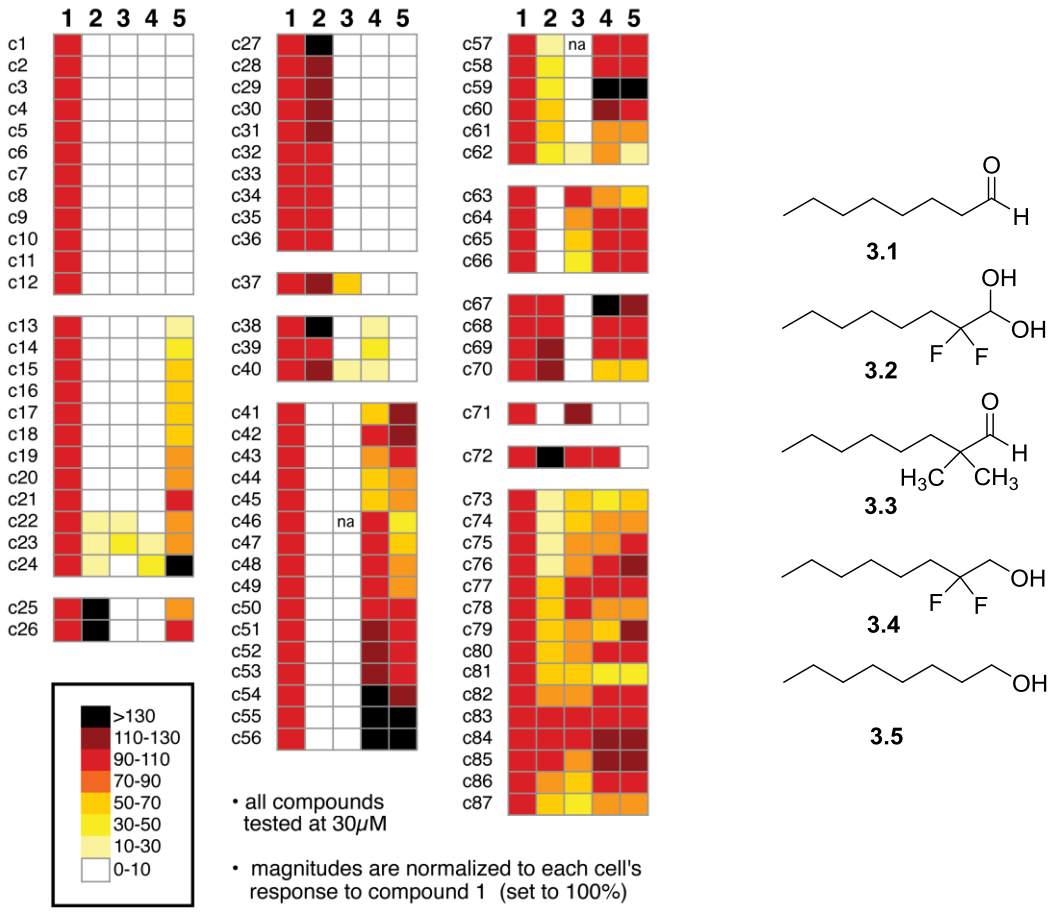


Figure 3.6 Summary of rat OSN responses to compounds **2.1**-**2.5**, as measured by calcium fluorescence imaging. (na, no data).

Non aldehyde specific receptors

Cells c13-c24 were activated by octanal and octanol, but not by 2,2-difluorooctanal, 2,2-dimethyloctanal and 2,2-difluorooctanol. These cells are very sensitive to the steric effect at C_2. Substitutions at C_2 cause the compounds to lose the ability to activate this group of ORs. This group therefore cannot give us any information on recognition of the hydrate by the ORs.

Cells c41-c56 were activated by octanal, octanol and 2,2-difluroooctanol, but not by 2,2-difluorooctanal and 2,2-dimethyloctanal. These cells are compatible for the 2,2-difluoro substitution, at least in the context of the alcohol. Addition of a second hydroxyl, in the context

of the gem-diol, was incompatible with activity, indicating they recognize the carbonyl form of the aldehyde.

Cells c57-c70 and c72-c87 are broadly tuned nonspecific receptors, and were activated by at least 4 compounds. c57-c62 and c67-c70 cannot tolerate the bulkiness of two methyl groups. c63-c66 cannot tolerate the second hydroxyl group of the hydrate. c73-c87 were activated by all five compounds. They may just recognize the features that all five compounds share, such as the size of the carbon chain and the presence of C_1 oxygen. This group cannot give us information on whether the hydrated form is recognized by any ORs.

Effect of 2,2-difluoro substitution on the recognition by ORs

The two fluorine group at C_2 can introduce steric effect and electron effect on the odorant. Comparing the cell responses from octanol and 2,2-difluorooctanol provide us some understating of the effect of 2,2-difluoro substitution. Out of the 59 cells that were able to recognize octanol, 45 cells (76%, c41-c70, c73-c87) were also activated by 2,2-difluorooctanol. Most of them show comparable potency by octanol and 2,2-difluorooctanol. This suggest that although the 2,2-difluor substitution dose remove the ability to activate by some ORs likely due to the steric effect, it is still largely compatible with most of the receptors. On the other hand, there were few cells (c72, weakly for c38-c40) that were not active by octanol but were activated by 2,2-diflourooctanol. Therefore, the 2,2-difluoro substitution only have limited negative effect on the potency of the compound and does not enhance the potency.(Figure 3.7b)

Checking the ability to from hydrate is an important strategy for a subset of aldehyde specific receptors

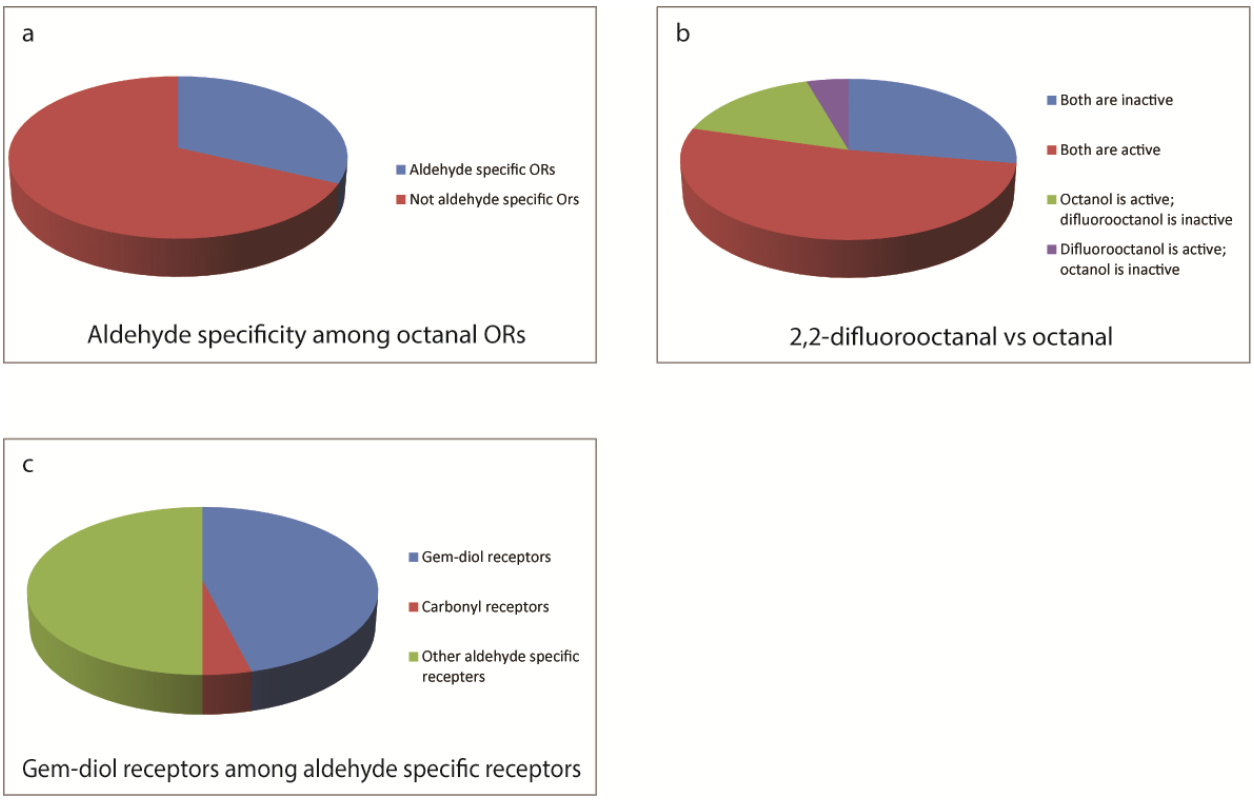


Figure 3.7 Data analysis of OSN responses result

The receptors that specifically recognize aldehydes, for example the rat OR-I7, are expected to be expressed by cells that were not activated by octanol (28 cells, 32%, c1-c12, c27-c40, c71-c72) but that are octanal-responsive. Cells c1-c10 were only activated by octanal and not by other 4 compounds. These cells are very specific for octanal, and are not compatible with the differences between octanal and other panel compounds.

Ten cells c27-c36 were activated by octanal and 2,2-difluorooctanal, but not by 2,2-dimethyloctanal, 2,2-difluorooctanol and octanol. *Comparing 2,2-difluorooctanal being active and 2,2-difluorooctanol being inactive suggests that the second hydroxyl group in hydrate is required for the activation of these ORs.* The cell response of these receptors for difluorooctanal was at least as strong as octanal. As discussed above, the 2,2-difluoro substitution affect barely

enhances the potency of the compounds. The higher potency of 2,2-difluorooctanal over octanal must is likely due to the higher content of the hydrate form of 2,2-difluorooctanal (~100%) over octanal (43%). These data are evidence that this subset of octanal receptors requires the gem-diol hydrate form for activation. c38-c40 also behaves similarly to c27-c38, and may also hydrated specific receptors. These 13 cells account for 46% of possible aldehyde-specific-receptors (Figure 3.7c). It should be noted that some of the cells in c1-c24 may also require gem-diol form for activation, but could not be observed due to the incompatibility with C-2 substitution.

The aldehyde recognition that requires the carbonyl form was not clearly observed. Only one cell c71 was activated by octanal and 2,2-dimethyloctanal, but not activated by 2,2-dimethyloctanal and the two alcohols. One of the reason could be that the two methyl groups of 2,2-dimethyloctanal are too bulky for larger amount of receptors than two fluorine, so some of the possible carbonyl receptors fall into c1-c12 group.

Kinetic of hydration

The hydration reaction can be catalyzed by acid or base. But in relatively neutral solutions, it takes several minutes to achieve the hydration equilibrium estimated from the hydration rate constant (k_0 = 3.5 x 10^{-3} s^{-1})[3b]. In the biological testing, this issue was addressed by leaving the aldehyde in buffer solution for enough amount of time to ensure the equilibrium had been reached. In reality, we can detect aldehydes with our nose much faster than few minutes. Since our result indicate that some aldehyde ORs require the gem-diol form for activation, the question arises how our nose finish it in such short amount of time. A possible explanation is that the nasal mucus dissolves different kinds of proteins which include an enzyme that catalyze the

hydration of aldehyde group. Alternatively, some aldehyde ORs may be able to catalyze the hydration.

3.4 Conclusion

Odorants are typically small hydrophobic molecules with one polar functional group. The hydrophobic carbon chain helps to keep the intermolecular interactions low so that the compound is volatile enough to vaporize and travel into our nose. Meanwhile, it lowers the solubility of the compound in the water-based nasal mucus. To compensate for this effect, nature might have developed a strategy for aldehyde recognition that is similar to the pro-drug strategy in pharmacology. Aldehydes travel as vapor in the carbonyl form, and once they reach the nose the formation of hydrates provide better hydrogen bond capacity for those gem-diol activated ORs. This may be especially important for smaller aldehydes, which have very little carbon skeleton for receptor to form van der Waals interactions with.

3.5 Experimental Procedure

General information

Materials

Commercial reagents and solvents were used without additional purification unless otherwise noted. 1-bromohexane and diethyl oxalate (Fisher Scientific); 1-Bromobutane (TCI America); solvents (VWR Intl.). THF and ether were dried and distilled from Na/benzophenone. All other reagents were from Sigma-Aldrich or Alfa Aesar. Reaction progress and chromatography fractions were monitored by thin layer chromatography (TLC) on silica gel coated glass plates.

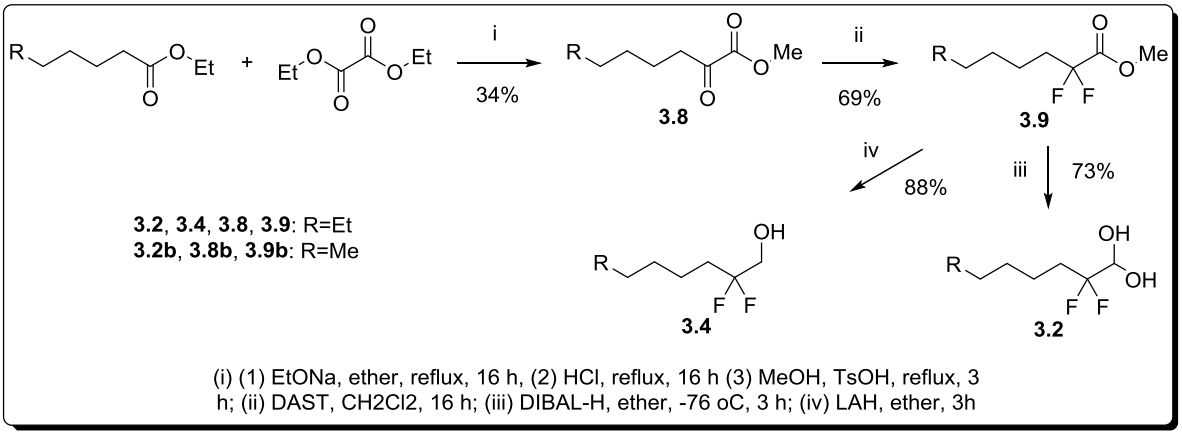
Flash chromatography purifications were performed using 230-400 mesh silica gel from Alfa Aesar. Freshly purified aldehydes were stored at -4 ºC under Argon and tested within 1 week.

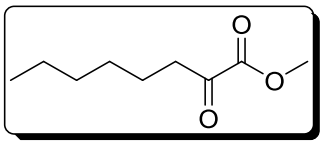
Compound characterization

NMR spectra were recorded on a Varian Mercury-300 spectrometer. Chemical shifts are reported in parts per million (ppm) referenced to the appropriate solvent peak. Infrared (IR) spectra were recorded using a Thermo Nicolet 6700 FT-IR spectrometer, and are reported in wavenumbers (cm^{-1}). GC/MS analyses were obtained on a Shimadzu GC/MS QP5000 with GC-17A Gas Chromatograph (capillary column: DB-1-30N-STD). Temperature programs for GC analysis: Program1: Injection temperature: 280 °C; held at 60 °C for 1min, heated from 60 to 200 °C at 5 °C/min, and held at 150 °C for 4 min; Program 2: Injection temperature: 280 °C; held at 60 °C for 4 min, heated from 60 to 280 °C at 20 °C/min, and held at 280 °C for 2 min. GC/MS of compound **3.6h**, **3.7h** and **3.3h** were obtained on Shimadzu GCMS-QP2010 with GC-2010 Plus Gas Chromatograph.

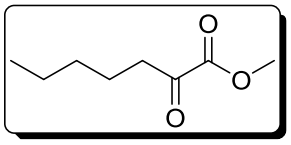
Synthetic procedures

2,2-Difluoroaldehydes and 2,2-difluorooctanol

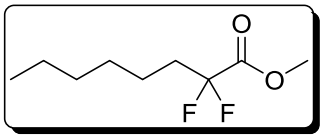




Methyl 2-ketooctanoate (3.8):[5] Sodium (4.39 g, 0.191 mol) was added to absolute ethanol (200 mL). After all the sodium was reacted, excess ethanol was removed under vacuum until a thick slurry was formed. Toluene (100 mL) was added and the mixture was evaporated under vacuum to remove residual ethanol. The resulting white solid was cooled and dry ether (100 mL) was added followed by diethyl oxalate (21.13 g, 0.145 mol). After all the NaOEt had dissolved, ethyl hexanoate (15 g, 0.095 mol) was added rapidly. The mixture was refluxed for 16 h, cooled and poured into water. The layers were separated. The ether layer was extracted twice with water. The aqueous extracts were combined and acidified with concentrated HCl and extracted with ether. The ether was evaporated and the residual oil was refluxed with concentrated HCl (50 mL) overnight. The mixture was cooled, diluted with ether and the two phases were separated. The aqueous layer was extracted with ether, and the combined organic layers were evaporated. The resulting oil was refluxed in methanol (150 mL) with a catalytic amount of TsOH for 3 h. The methanol was evaporated and the residue was diluted with ether and extracted with 20% potassium carbonate solution. The ether solution was dried and evaporated. The crude product was chromatographed with 10% EtOAc and 90% hexanes to give 5.5 g (34%) of keto ester **3.8**. Spectral data matched those reported previously[8].

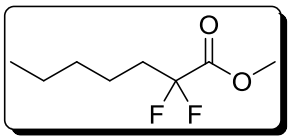


Methyl 2-ketoheptanoate (3.8b): Same procedure as for **3.8.** Spectral data match those reported previously[8].

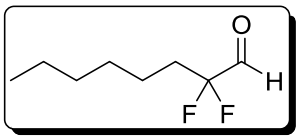


Methyl 2,2-difluorooctanoate (3.9):[5] To an ice-cooled solution of methyl 2-ketooctanoate **3.8** (5 g, 0.029 mmol) in CH_2Cl_2 (40 mL) was added dropwise diethylaminosulfur trifluoride (DAST; 5 g, 0.031 mmol). The mixture was stirred at room temperature overnight. Crushed ice was added followed by $NaHCO_3$ to neutralize the aqueous

layer. The layers were separated. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with H_2O and dried with $MgSO_4$. The solvent was evaporated and the crude product was chromatographed with 20% CH_2Cl_2 and 80% hexane to give 3.9 g (69%) difluoro ester. Spectral data match those reported previously[9].

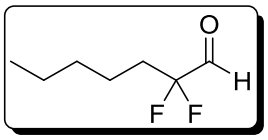


Methyl 2,2-difluoroheptanoate (3.9b): Same procedure as for **3.9.** ^{1}H NMR ($CDCl_3$,300MHz): δ 3.88 (s, 3 H), 1.94 - 2.17 (m, 2 H), 1.40 - 1.54 (m, 2 H), 1.25 - 1.40 (m, 4 H), 0.90 (t, *J*=6.7 Hz, 3 H); ^{13}C NMR ($CDCl_3$,75MHz): δ 164.9 (t, *J*=33.7 Hz), 116.4 (t, *J*=249.3 Hz), 53.2 (q, *J*=24.47 Hz), 34.4 (t, *J*=22.7 Hz), 31.1, 22.2, 21.1, 13.8; ^{19}F NMR (282MHz ,$CDCl_3$) δ -106.2 (t, *J*=15.3 Hz); IR (neat), ν: 2960, 2936, 2875, 1765, 1443, 1348, 1303, 1246, 1198, 1151, 1124, 1067, 1036, 949, 823, 779, 730; GC retention time: 10.01 min (program 1); MS (EI): 165 $(M\text{-}CH_3)^+$.

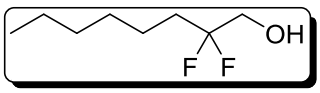


2,2-difluorooctanal (3.2)[5]: To a solution of methyl 2,2-difluorooctanoate **3.9** (0.55 g, 2.83 mmol) in ether (8 mL), cooled to -78 °C, was added dropwise over 30 min 25% diisobutylaluminum hydride in toluene (2.76 mL, 3.40 mmol). The mixture was stirred at -78 °C for 2 h (monitored by GC/MS) and slowly warmed up to 0 °C over 3h. While at 0 ºC, ether was added to dilute the mixture followed by addition of 4M H_2SO_4. The aqueous layer was extracted with ether and the combined organic layers were washed with $NaHCO_3$ solution, brine and dried. The solvent was evaporated and the crude product was chromatographed with 50% ether and 50% hexane, to give 0.34 g (73%) difluoro aldehyde. ^{1}H NMR (D_2O ,300MHz): δ 5.06 (t, *J*=7.6 Hz, 1 H), 1.77 - 2.06 (m, 2 H), 1.40 - 1.56 (m, 2 H), 1.17 - 1.40 (m, 6 H), 0.84 (t, *J*=6.7 Hz, 3 H); GC retention time: 5.34 min (program 1); MS (EI): 149 $(M\text{-}CH_3)^+$, 135 $(M\text{-}CHO)^+$; **2,4-DNP derivative** (see also the note in paragraph after next): 50 mg of product was mixed with DNP in 95% ethanol with concentrated

H_2SO_4. The resulting solid was filtered and recrystallized from 95% ethanol. ^{1}H NMR ($CDCl_3$,300MHz): δ 11.20 (s, 1 H), 9.13 (d, *J*=2.3 Hz, 1 H), 8.33 - 8.48 (dd, *J*=9.7, 2.1 Hz, 1 H), 7.94 (d, *J*=9.4 Hz, 1 H), 7.53 (t, *J*=4.1 Hz, 1 H), 2.08 - 2.33 (m, 2 H), 1.51 - 1.70 (m, 2 H), 1.23 - 1.51 (m, 6 H), 0.91 (t, *J*=6.7 Hz, 3 H); ^{13}C NMR ($CDCl_3$,75MHz): δ 144.5, 142.0 (t, *J*=37.1 Hz), 139.4, 130.4, 130.2, 123.1, 119.7 (t, *J*=237.5 Hz), 116.8, 34.8 (t, *J*= 23.6 Hz), 31.5, 29.0, 22.4, 21.7, 14.0 ; IR (neat), ν: 3305, 3092, 2951, 2930, 2870, 2862, 1616, 1589, 1520, 1509, 1425, 1238, 1224, 1177, 1135, 1084, 1059, 1012, 965, 927, 902, 867, 831, 808, 741, 689; melting point: 74-75 ºC.



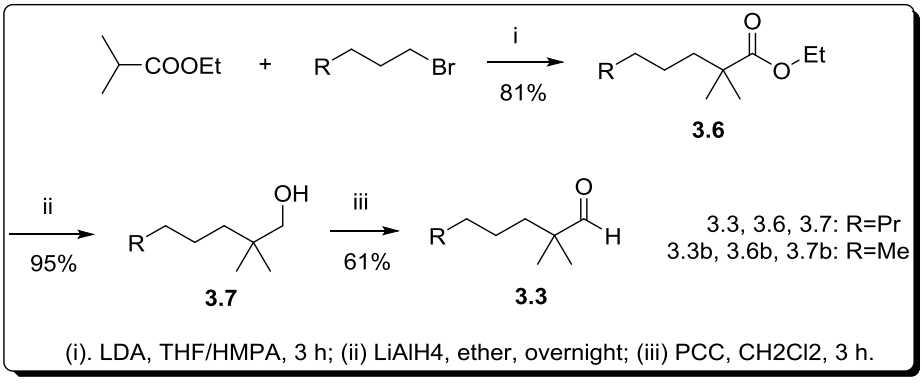
2,2-difluoroheptanal (3.2b) Same procedure as for **3.2.** ^{1}H NMR (D_2O ,300MHz): δ 5.08 (t, *J*=7.6 Hz, 1 H), 1.80 - 2.08 (m, 2 H), 1.49 (d, *J*=7.0 Hz, 2 H), 1.18 - 1.41 (m, 4 H), 0.75 - 0.98 (m, 3 H); MS (EI): 135 $(M\text{-}CH_3)^+$, 121 $(M\text{-}CHO)^+$; GC retention time: 4.15 min (program 2); 2,4-DNP derivative: ^{1}H NMR ($CDCl_3$,300MHz): δ 11.20 (s, 1 H), 9.13 (d, *J*=2.6 Hz, 1 H), 8.39 (dd, *J*=9.5, 2.5 Hz, 1 H), 7.94 (d, *J*=9.7 Hz, 1 H), 7.54 (t, *J*=4.3 Hz, 1 H), 2.07 - 2.36 (m, 2 H), 1.50 - 1.73 (m, 2 H), 1.26 - 1.50 (m, 4 H), 0.93 (t, *J*=6.7 Hz, 3 H); ^{13}C NMR ($CDCl_3$,75MHz): δ 144.8, 142.5 (t, *J*=37.4 Hz), 139.6, 130.6, 130.4, 123.4, 120.0 (t, *J*=237.1 Hz), 117.0, 34.9 (t, *J*=24.1 Hz), 31.7, 22.7, 21.7, 14.2 ; IR (neat), ν: 3297, 3108, 2961, 2938, 2871, 1615, 1591, 1505, 1426, 1321, 1277, 1225, 1135, 1078, 1045, 938, 927, 834, 741, 667; melting point: 88-91 ºC.

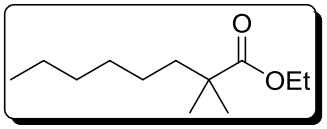


2,2-difluorooctanol (3.4) To a mixture of $LiAlH_4$ (50 mg, 1.32 mmol) and ether (1.5 mL) cooled to 0 ºC, was added methyl 2,2-difluorooctanoate (0.21 g, 1.1 mmol) in ether (0.5 mL). The mixture was stirred at room temperature for 2 h and re-cooled to 0 ºC. The mixture was worked up by dropwise and sequential addition of 0.05 mL H_2O, 0.05 mL 15% NaOH solution, 0.15 mL H_2O and then

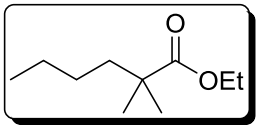
stirred for 5 min. The mixture was filtered and washed with ether. The resulting solution was dried with $MgSO_4$ and solvent was evaporated to give 0.16 g (88%) product. Spectral data matched those reported previously[10].

2,2-dimethyloctanal (3) and 2,2-dimethylhexanal (for ^{1}H-NMR)



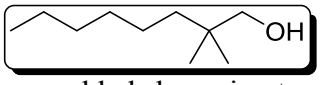


Ethyl 2,2-dimethyloctanoate (3.6): 1.6 M *n*-BuLi solution in THF (5.38 mL, 8.61 mmol) was added to diisopropylamine (1.36 mL, 9.73 mmol) in 15 mL dry THF at -75 °C. The mixture was stirred for 1 h. Ethyl isobutyrate (1 g, 8.61 mmol) was added with stirring. After 30 min, 1-bromohexane (1.32 mL, 9.47 mmol) in HPMA (1.6 mL) was added. The reaction was stirred at -78 °C for 1 h and allowed to warm slowly to room temperature over a period of 2 h. The reaction was quenched with water (0.5 mL), stirred briefly then dried with $MgSO_4$, and the solvent was evaporated. The crude product was chromatographed on silica gel with 5% ether and 95% hexane to give 1.4 g (81%) of **3.6**. Spectral data match those reported previously[11].



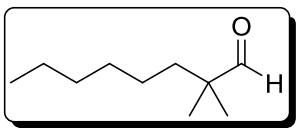
Ethyl 2,2-dimethylhexanoate (3.6b): Same procedure as for **3.6** prepared from ethyl isobutyrate and 1-bromobutane. ^{1}H NMR ($CDCl_3$,300MHz): δ 4.10 (q, *J*=7.2 Hz, 2 H), 1.45 - 1.55 (m, 2 H), 1.17 - 1.36 (m, 7 H), 1.14 (s, 7 H), 0.88 (t, *J*=7.2 Hz, 3 H); ^{13}C NMR ($CDCl_3$,75MHz): δ 178.1, 60.1, 42.1, 40.4, 27.1, 25.1, 23.1, 14.2, 14.0 ; IR

(neat), ν: 2959, 2935, 1728, 1473, 1275, 1203, 1147, 1095, 1029; GC retention time: 10.242 min; MS (EI): 57 (100), 88 (17), 99 (25), 116 (39).



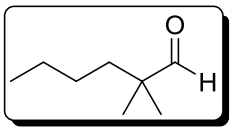
2,2-dimethyloctanol (3.7): 3.6 (1.2 g, 6.99 mmol) in 5 mL dry ether was added dropwise to an ice-cold mixture of LAH (0.273 g, 7.2 mmol) in 15 mL ether with stirring. The mixture was stirred at room temperature overnight and then re-cooled to 0 °C. The mixture was worked up by dropwise and sequential addition of 0.27 mL H_2O, 0.27 mL 15% NaOH solution, 0.81 mL H_2O and then stirred for 5 min. The mixture was filtered through a celite pad and extracted with ether. The ether extract was dried with $MgSO_4$ and the solvent was evaporated to give 0.9 g (95%) alcohol product. ^{1}H NMR ($CDCl_3$,300MHz): δ 3.32 (d, *J*=6.1 Hz, 2 H), 1.04 - 1.39 (m, 11 H), 0.76 - 0.96 (m, 9 H); ^{13}C NMR ($CDCl_3$,75MHz): δ 72.1, 38.7, 35.0, 31.9, 30.3, 23.8, 22.7, 14.1 ; IR (neat), ν: 3346, 2928, 2859, 1469, 1380, 1364, 1044, 724; GC retention time: 12.36 min (program 1); MS (EI): 127 (M-CH_2OH^+, 18), 57(100).

2,2-dimethylhexanol (3.7b): Same procedure as for **3.7** prepared from **3.6h**. ^{1}H NMR ($CDCl_3$, 300MHz): δ 3.30 (s, 2 H), 1.66 (br. s., 1 H), 1.14 - 1.36 (m, 6 H), 0.89 (t, *J*=6.9 Hz, 3 H), 0.85 (d, *J*=1.2 Hz, 6 H); ^{13}C NMR ($CDCl_3$, 75MHz): δ 72.0, 38.4, 34.9, 26.1, 23.6, 23.8, 14.1 ; IR (neat), ν: 3343, 2955, 2929, 2861, 1469, 1380, 1364, 1039, 728; GC retention time: 8.640 min; MS (EI): 99 (M-$CH_2OH)^+$.



2,2-dimethyloctanal (3.3): A solution of 2,2-dimethyloctanol (0.25 g, 1.58 mmol) in dry CH_2Cl_2 (2.5 mL) was added to PCC (0.51 g, 2.37 mmol) and silica gel (0.25 g) in dry CH_2Cl_2 (2.5 mL) with stirring. After stirring for 3 h, the mixture was filtered through a silica gel pad and washed with excess CH_2Cl_2. The yellow liquid from evaporation of CH_2Cl_2 was chromatographed on silica gel with 40% CH_2Cl_2 and 60%

hexanes to give 0.15 g (61%) aldehyde **3**. ^{1}H NMR ($CDCl_3$, 300MHz): δ 9.44 (s, 1 H), 1.41 - 1.49 (m, 2 H), 1.12 - 1.33 (m, 8 H), 1.04 (s, 6 H), 0.88 (t, *J*=6.4 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 75MHz): δ 206.7, 45.8, 37.3, 31.6, 29.9, 24.2, 22.6, 21.3, 14.0 ; IR (neat), ν: 2959, 2931, 2859, 2801, 2779, 2690, 1728, 1469, 1396, 1378, 1367, 1192, 1154, 904, 884, 771, 724; GC retention time: 8.44 min (program 2); MS (EI): 127 $(M\text{-}CHO)^{+}$.



2,2-dimethylhexanal (3.3b): Same procedure as for **3.3**. ^{1}H NMR ($CDCl_3$, 300MHz): δ 9.45 (s, 1 H), 1.38 - 1.58 (m, 2 H), 1.13 - 1.38 (m, 4 H), 1.04 (s, 6 H), 0.89 (t, *J*=7.0 Hz, 3 H); ^{13}C NMR ($CDCl_3$,75MHz): δ 206.5, 45.7, 37.0, 26.4, 23.3, 21.2, 13.9 ; IR (neat), ν: 2959, 2932, 2862, 1727, 1470, 1379, 1366, 885, 770, 729; GC retention time: 7.053 min; MS EI: 99 ($M\text{-}CHO^{+}$, 7), 72 (25), 57 (100).

Equilibria studies using ^{1}H-NMR:

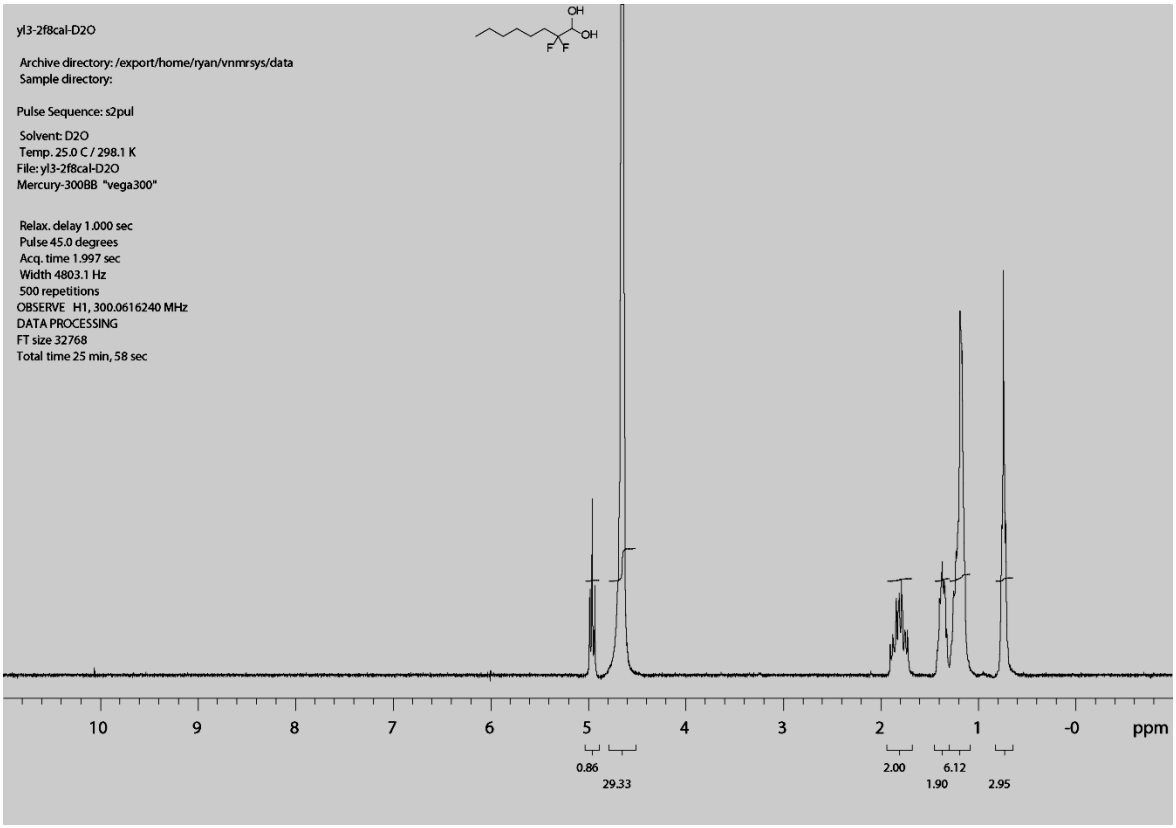
Hydration: 3 mg of hexanal or difluoroheptanal were dissolved in 1 mL D_2O. 64 transients were taken. For dimethylhexanal, ~0.5 mg was used because of its lower solubility in water, and 800 transients were accumulated. The equilibrium ratio was measured 10 min after dissolving.

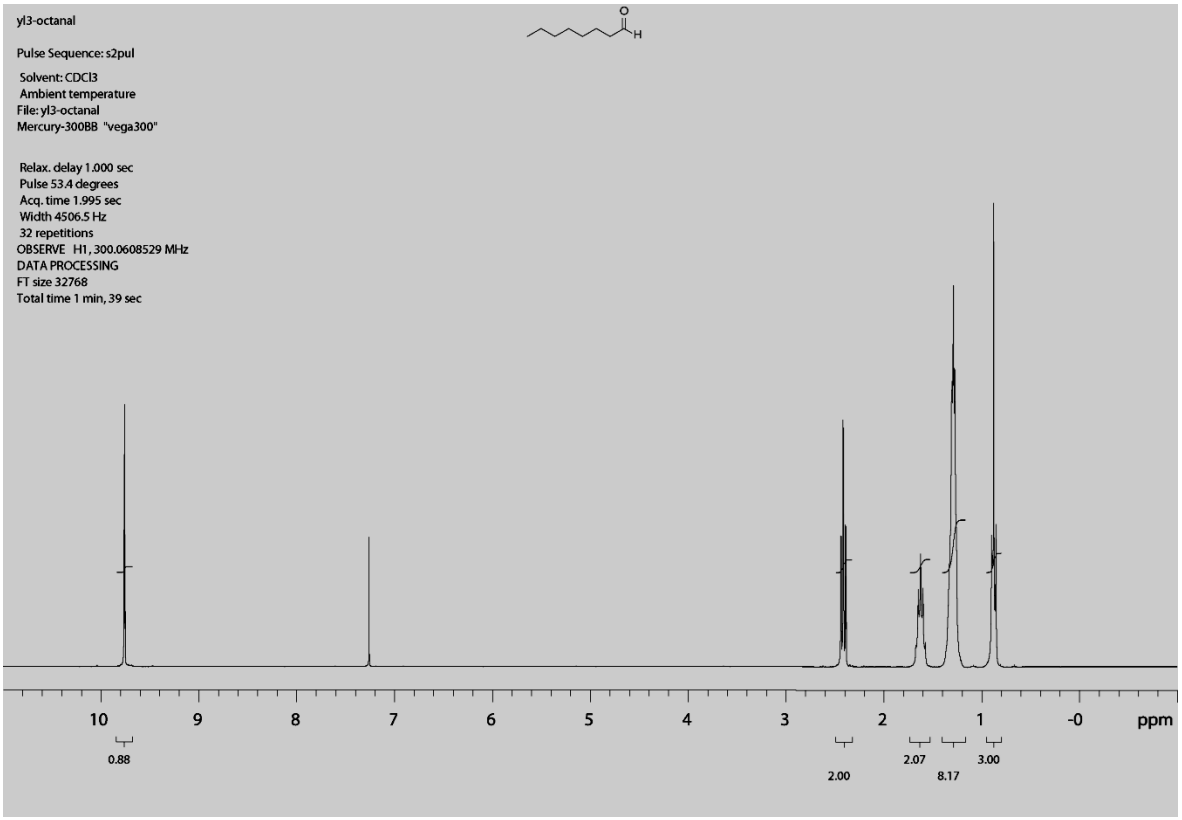
Methyl acetal formation: 3 mg aldehyde and 30 molar equiv. CD_3OD were dissolved in 1 mL D_2O. 64 transients were taken. The equilibrium ratio and spectrum shown were taken 60 min after mixing, after which no further change could be observed.

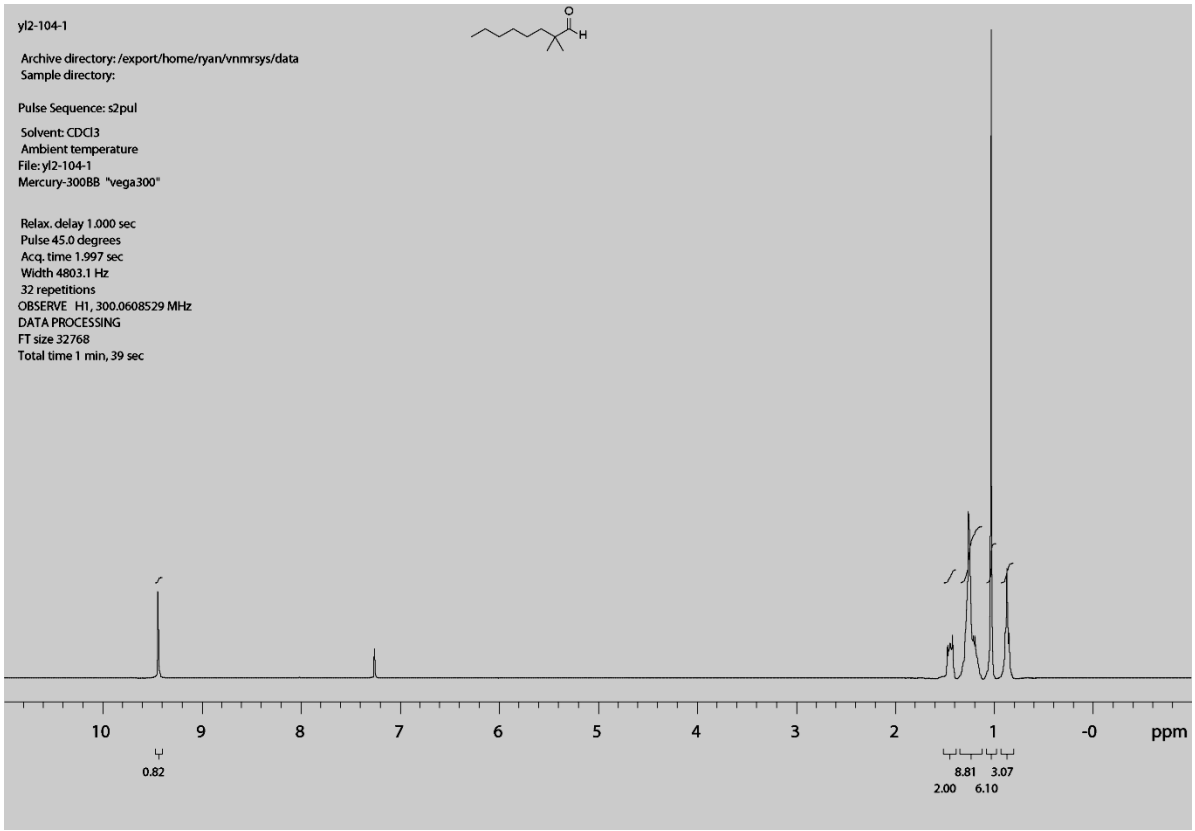
Schiff base formation : 3 mg aldehyde and 0.3 molar equiv. ethanolamine were dissolved in 1 mL D_2O. 64 transients were taken. The equilibrium ratio and spectrum shown were taken 30 min after mixing, after which no further change could be observed.

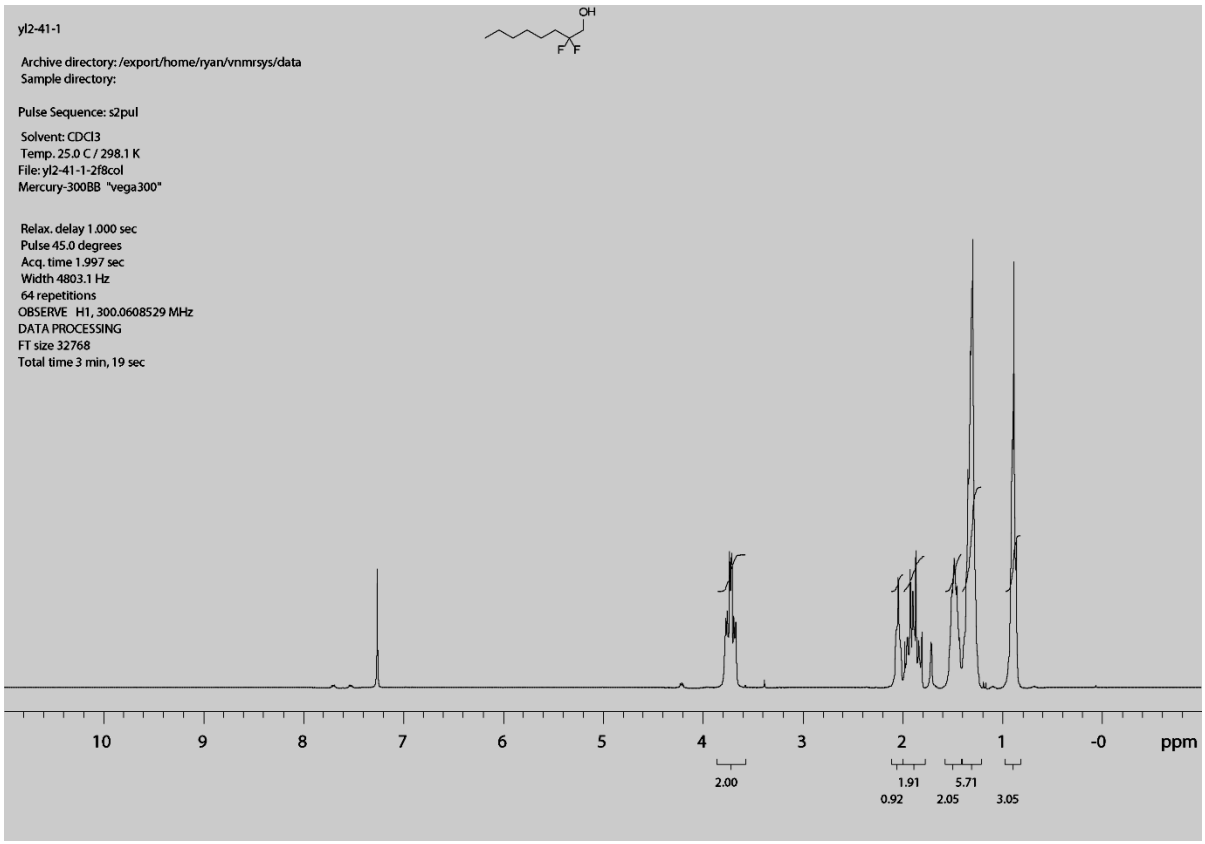
Organoleptic observations on difluoroaldehydes. During the course of the syntheses it was noticed that 2,2-difluorooctanal had a unique citrus-watermelon smell. The 2,2-difluoroheptanal was noticed to have a pronounced green smell, like fresh-mown grass. 2,2-Difluoro substitution prevented air oxidation of the aldehydes; the smell of these compounds kept in a closed screw-cap vial under air was stable for at least 2 years, whereas octanal took on the smell of octanoic acid within a week of air exposure when stored identically.

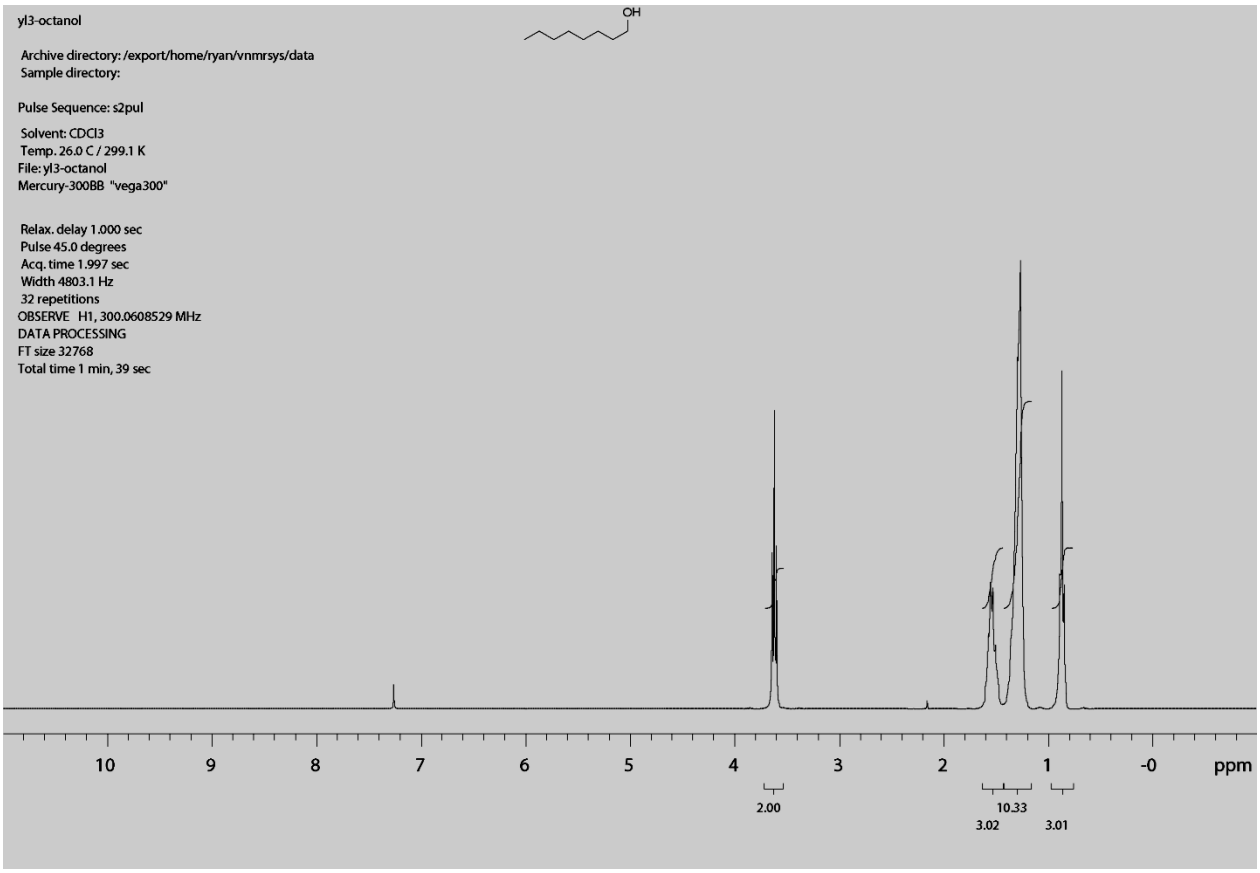
^{1}H-NMR spectra of final tested panel compounds:



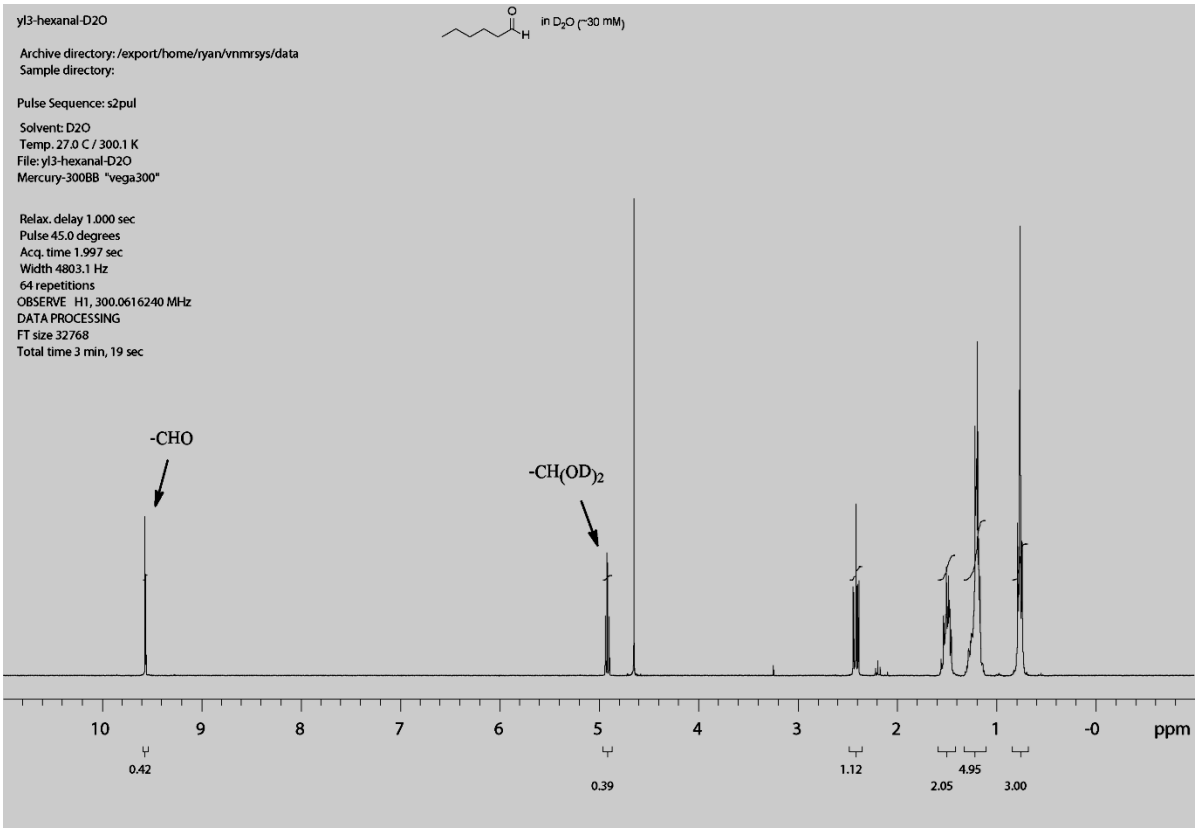

yl3-octanal
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: yl3-octanal
Mercury-300BB "vega300"
Relax. delay 1.000 sec
Pulse 53.4 degrees
Acq. time 1.995 sec
Width 4506.5 Hz
32 repetitions
OBSERVE H1, 300.0608529 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 39 sec
O
H
10
9
8
7
6
5
4
3
2
1
-0
ppm
0.88
2.00
2.07
8.17
3.00

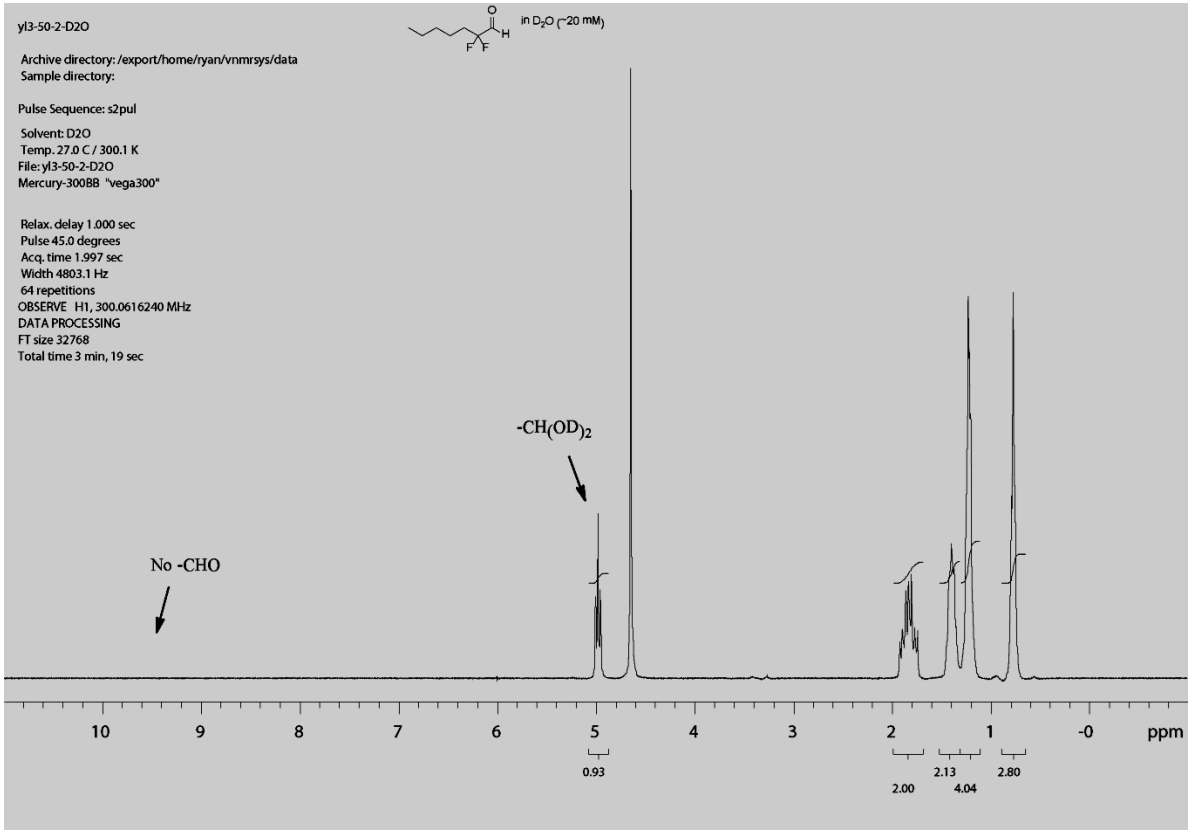

yl2-104-1
Archive directory: /export/home/ryan/vnmrsys/data
Sample directory:
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: yl2-104-1
Mercury-300BB "vega300"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.997 sec
Width 4803.1 Hz
32 repetitions
OBSERVE H1, 300.0608529 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 39 sec
O
H
10
9
8
7
6
5
4
3
2
1
-0
ppm
0.82
8.81
3.07
2.00
6.10

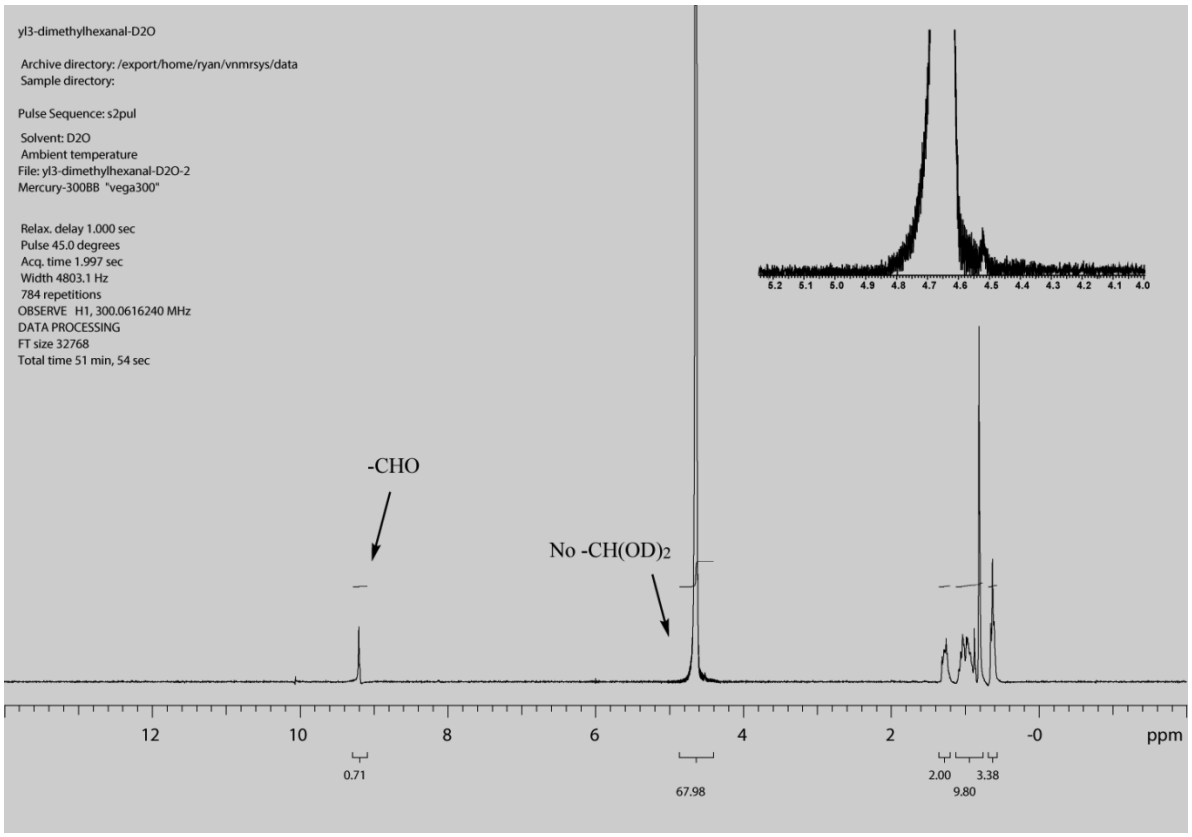

yl2-41-1
Archive directory: /export/home/ryan/vnmrsys/data
Sample directory:
Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
File: yl2-41-1-2f8col
Mercury-300BB "vega300"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.997 sec
Width 4803.1 Hz
64 repetitions
OBSERVE H1, 300.0608529 MHz
DATA PROCESSING
FT size 32768
Total time 3 min, 19 sec
OH
F
F
10
9
8
7
6
5
4
3
2
1
-0
ppm
2.00
1.91
0.92
5.71
2.05
3.05

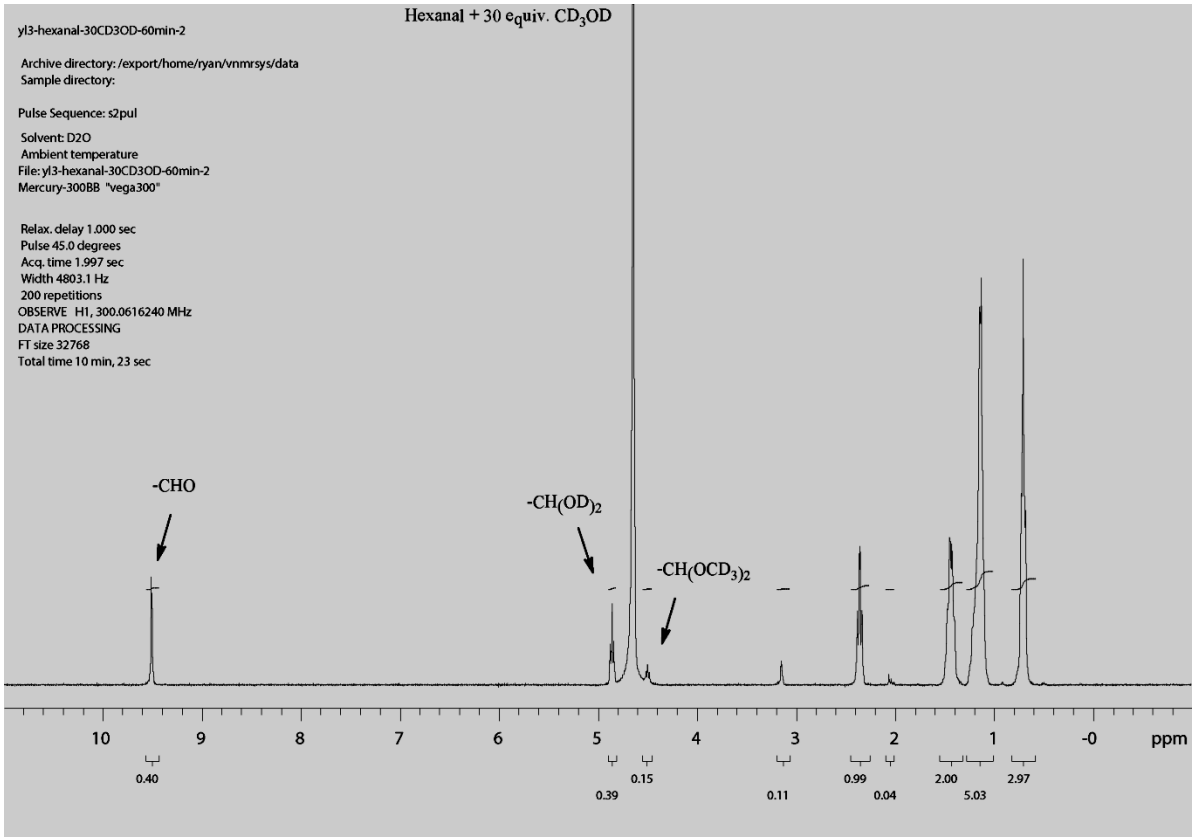


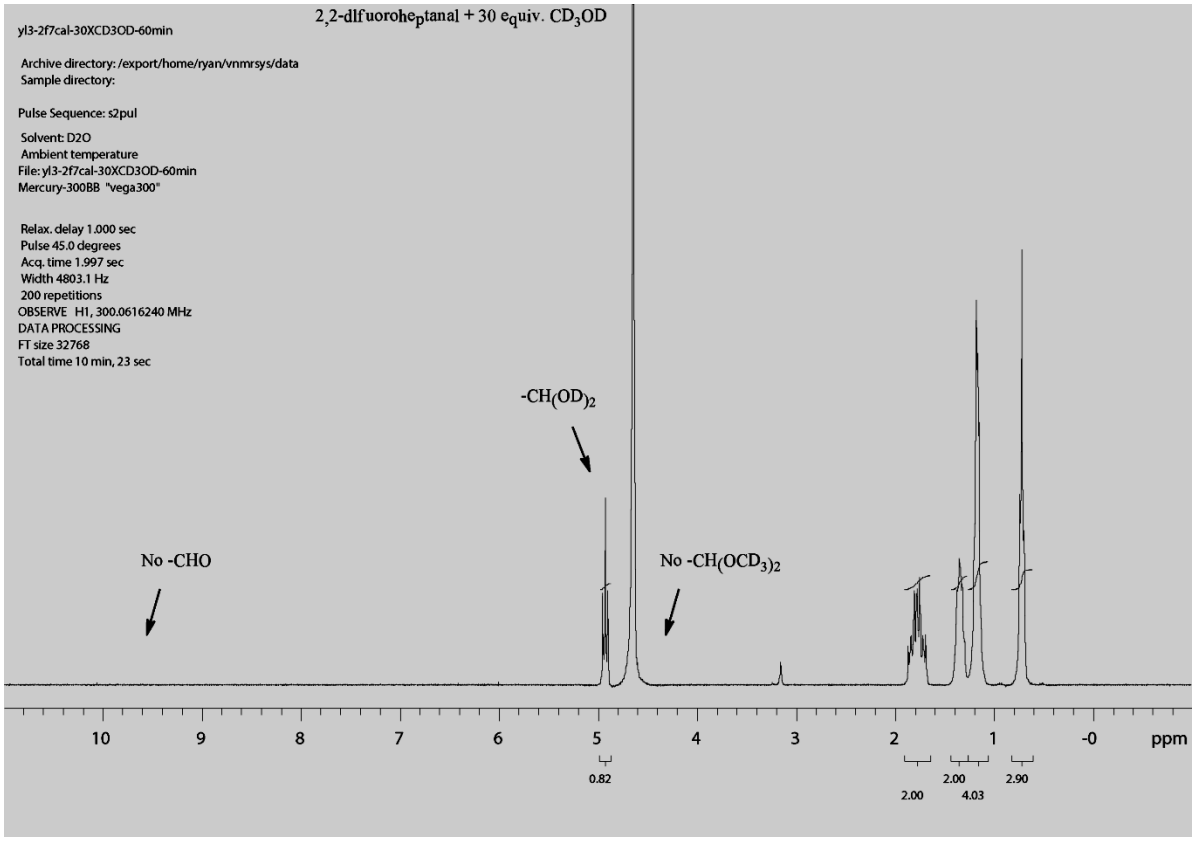
^{1}H-NMR spectra used to measure aldehyde equilibria:

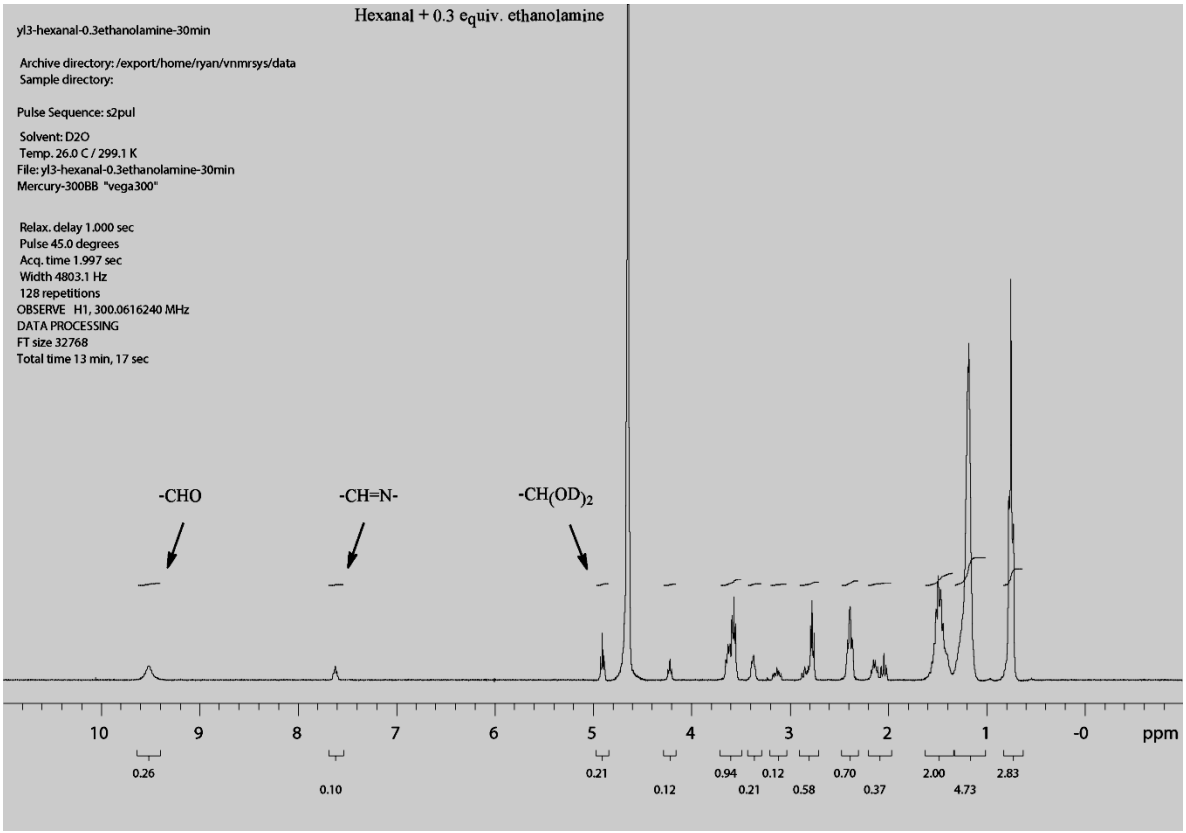

yl3-hexanal-D2O
in D2O (~30 mM)
Archive directory: /export/home/ryan/vnmrsys/data
Sample directory:
Pulse Sequence: s2pul
Solvent: D2O
Temp. 27.0 C / 300.1 K
File: yl3-hexanal-D2O
Mercury-300BB "vega300"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.997 sec
Width 4803.1 Hz
64 repetitions
OBSERVE H1, 300.0616240 MHz
DATA PROCESSING
FT size 32768
Total time 3 min, 19 sec
-CHO
-CH(OD)2
10 9 8 7 6 5 4 3 2 1 -0 ppm
0.42
0.39
1.12
4.95
2.05
3.00

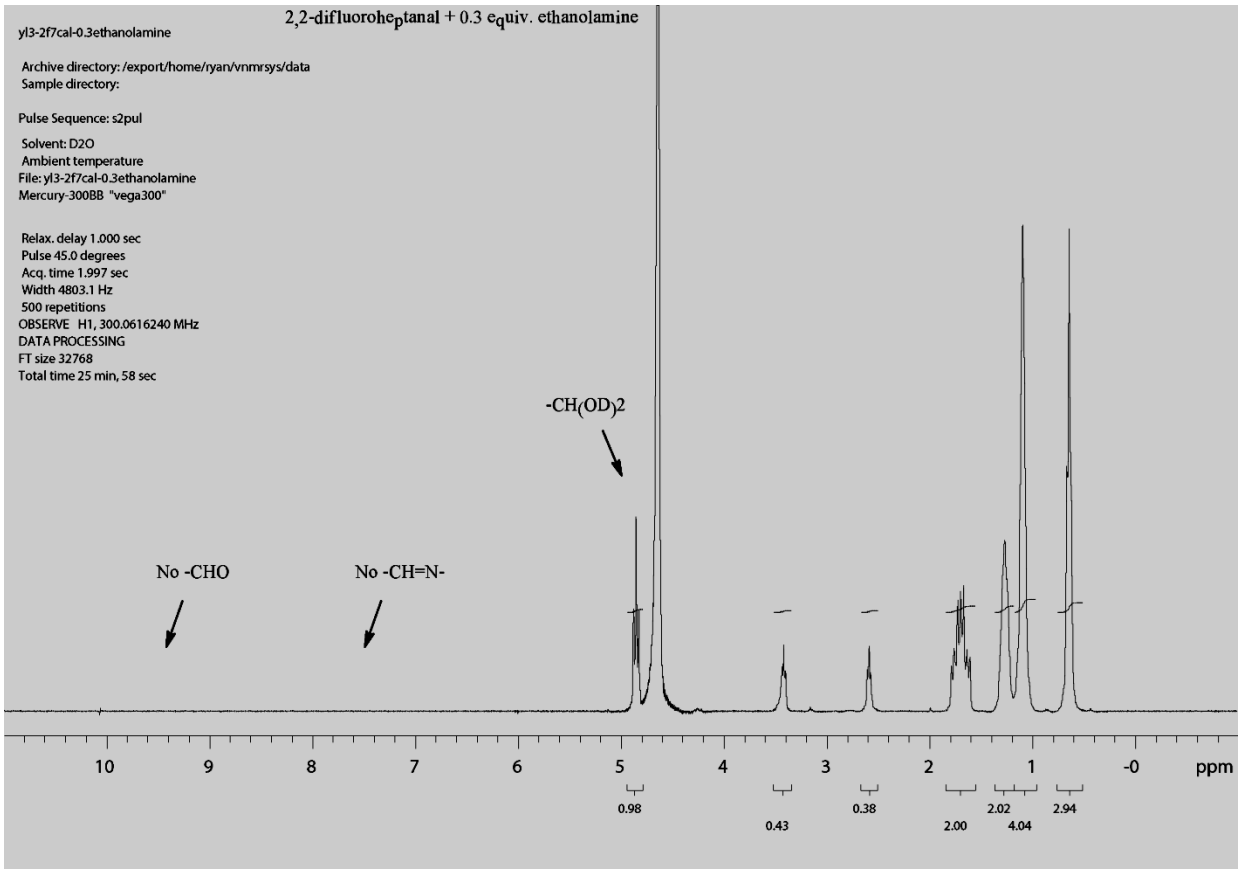

yl3-50-2-D2O
in D2O (~20 mM)
Archive directory: /export/home/ryan/vnmrsys/data
Sample directory:
Pulse Sequence: s2pul
Solvent: D2O
Temp. 27.0 C / 300.1 K
File: yl3-50-2-D2O
Mercury-300BB "vega300"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.997 sec
Width 4803.1 Hz
64 repetitions
OBSERVE H1, 300.0616240 MHz
DATA PROCESSING
FT size 32768
Total time 3 min, 19 sec
-CH(OD)2
No -CHO
10 9 8 7 6 5 4 3 2 1 -0 ppm
0.93
2.00
2.13
4.04
2.80


yl3-dimethylhexanal-D2O
Archive directory: /export/home/ryan/vnmrsys/data
Sample directory:
Pulse Sequence: s2pul
Solvent: D2O
Ambient temperature
File: yl3-dimethylhexanal-D2O-2
Mercury-300BB "vega300"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.997 sec
Width 4803.1 Hz
784 repetitions
OBSERVE H1, 300.0616240 MHz
DATA PROCESSING
FT size 32768
Total time 51 min, 54 sec
-CHO
No -CH(OD)2
12
10
8
6
4
2
-0
ppm
0.71
67.98
2.00
3.38
9.80


Hexanal + 30 equiv. CD3OD
yl3-hexanal-30CD3OD-60min-2
Archive directory: /export/home/ryan/vnmrsys/data
Sample directory:
Pulse Sequence: s2pul
Solvent: D2O
Ambient temperature
File: yl3-hexanal-30CD3OD-60min-2
Mercury-300BB "vega300"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.997 sec
Width 4803.1 Hz
200 repetitions
OBSERVE H1, 300.0616240 MHz
DATA PROCESSING
FT size 32768
Total time 10 min, 23 sec
-CHO
-CH(OD)2
-CH(OCD3)2
10
9
8
7
6
5
4
3
2
1
-0
ppm
0.40
0.39
0.15
0.11
0.99
0.04
2.00
5.03
2.97


2,2-dlfuoroheptanal + 30 equiv. CD3OD
yl3-2f7cal-30XCD3OD-60min
Archive directory: /export/home/ryan/vnmrsys/data
Sample directory:
Pulse Sequence: s2pul
Solvent: D2O
Ambient temperature
File: yl3-2f7cal-30XCD3OD-60min
Mercury-300BB "vega300"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.997 sec
Width 4803.1 Hz
200 repetitions
OBSERVE H1, 300.0616240 MHz
DATA PROCESSING
FT size 32768
Total time 10 min, 23 sec
-CH(OD)2
No -CHO
No -CH(OCD3)2
10 9 8 7 6 5 4 3 2 1 -0 ppm
0.82
2.00
2.00
4.03
2.90


Hexanal + 0.3 equiv. ethanolamine
yl3-hexanal-0.3ethanolamine-30min
Archive directory: /export/home/ryan/vnmrsys/data
Sample directory:
Pulse Sequence: s2pul
Solvent: D2O
Temp. 26.0 C / 299.1 K
File: yl3-hexanal-0.3ethanolamine-30min
Mercury-300BB "vega300"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.997 sec
Width 4803.1 Hz
128 repetitions
OBSERVE H1, 300.0616240 MHz
DATA PROCESSING
FT size 32768
Total time 13 min, 17 sec
-CHO
-CH=N-
-CH(OD)2
10 9 8 7 6 5 4 3 2 1 -0 ppm
0.26
0.10
0.21
0.12
0.94
0.21
0.12
0.58
0.70
0.37
2.00
4.73
2.83


2,2-difluoroheptanal + 0.3 equiv. ethanolamine
yl3-2f7cal-0.3ethanolamine
Archive directory: /export/home/ryan/vnmrsys/data
Sample directory:
Pulse Sequence: s2pul
Solvent: D2O
Ambient temperature
File: yl3-2f7cal-0.3ethanolamine
Mercury-300BB "vega300"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.997 sec
Width 4803.1 Hz
500 repetitions
OBSERVE H1, 300.0616240 MHz
DATA PROCESSING
FT size 32768
Total time 25 min, 58 sec
-CH(OD)2
No -CHO
No -CH=N-
10
9
8
7
6
5
4
3
2
1
-0
ppm
0.98
0.43
0.38
2.00
2.02
4.04
2.94

Chapter 4: Additional Hypothesis-based Octanal Analogues: Design, Synthesis and Biological Data

4.1 Introduction

There were other octanal analogues that were designed, synthesized and tested. These compounds test how certain conformation (compound **4.1**, **4.2** and **4.3**), hydrogen bonding ability and dipole-dipole interactions (compound **4.4**) and hydration (compound **4.5**, **4.6**, **4.7** and **4.8**) influence the recognition of octanal by rat OR-I7. It was not possible to draw a clear conclusion from these compounds, as the results could be interpreted in different ways. But they provide some additional information for what was discussed in previous chapters, and may serve as useful guide for future studies. These octanal analogues are summarized in Figure 4.1. Their design, synthesis and testing will be discussed in this chapter.

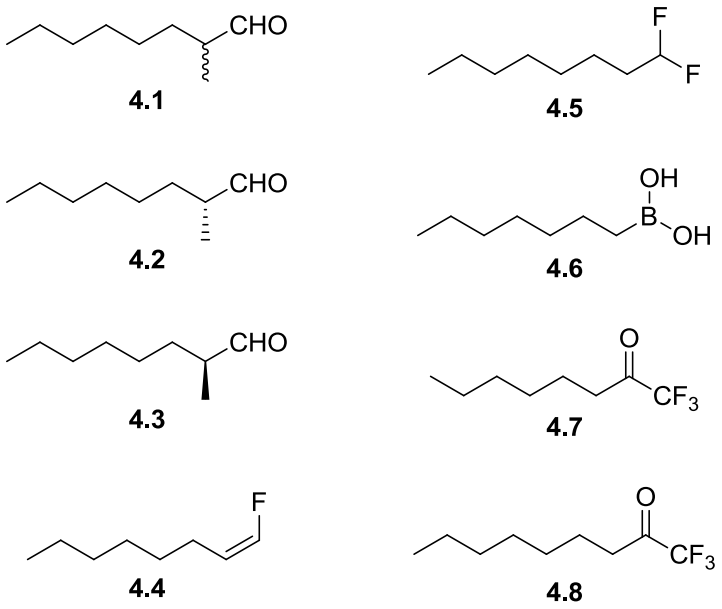


Figure 4.1 Summary of miscellaneous octanal analogues

4.2 Result

The effect of C_2 substitution on the activity of octanal

The enantiomers of many odorant molecules differ in strength and in odor description[1]. Olfactory receptors are proteins that are built from chiral amino acids. Enantiomers can interact differently with olfactory receptors resulting in different potencies. 2-methyloctanal was designed to test if and how OR-I7 distinguishes enantiomers. Its homolog 2-methylundecanal, is one of the first synthetic aldehyde to be used in the famous aldehydic perfume Chanel No. 5, and is responsible for the signature scent.

Cycloheptylformaldehyde (compound **6** in chapter 1, Figure 4.2) with one alkyl group at sp^3 C_2 was able to bind to OR-I7 as an antagonist, suggesting that OR-I7 can tolerate one alkyl substitution at C_2. However, two methyl groups substitution at C_2 seems to be not tolerated by OR-I7, as 2,2-dimethyloctanal (compound **3** in chapter 3, Figure 4.2) was found in preliminary tests by the Firestein lab to be a very low potency agonist. Previous testing by Prof. Firestein's group[2] also showed evidence of structural constraints near the aldehyde group. 2,5,7-trimethyl-2-octenal(Figure 4.2) was found inactive and unable to bind to OR-I7 because the sp^2 C_2 methyl group was forced into the plane of carbonyl and C_2=C_3 bond. Therefore, the space where the C_2 substitution is accommodated is unlikely to be in the plane of the carbonyl group, i.e., the binding pocket near the carbonyl group is unsymmetrical with respect to the enantiomer methyl position. If we assume the orientation of the aldehyde group is fixed within OR-I7, then forcing the two C_2 methyl groups in compound **4.2** and **4.3** into the same

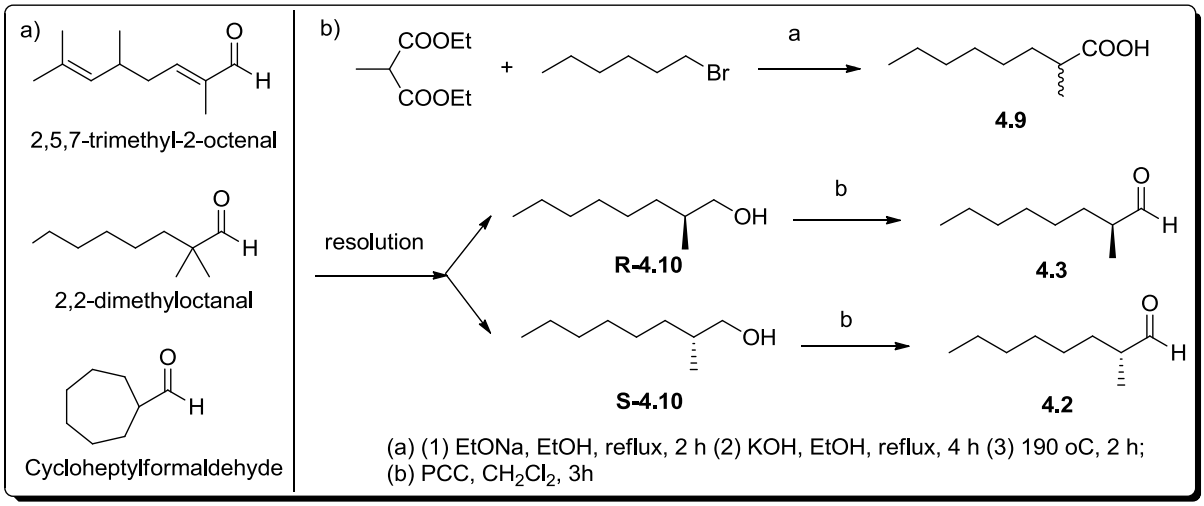


Figure 4.2 Synthesis of (R)-2-methyloctanal and (s)-2-methyloctanal

position would at the same time force the long alkyl chains to adopt different orientation and result in different potency of the two enantiomers.

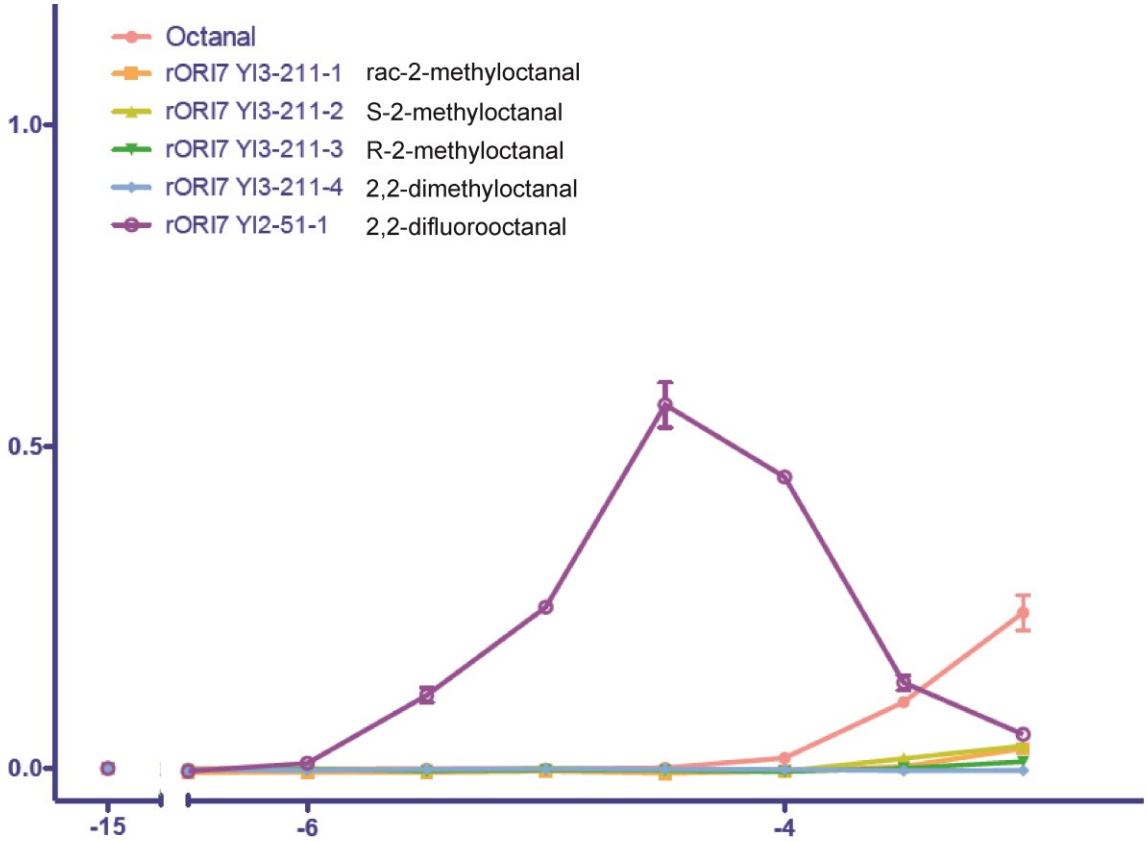


Figure 4.3 Activation dose-response curves of 2-methyloctanal

The two enantiomers and racemic mixture were synthesized as shown in Figure 4.2. The racemic acid **4.9** was resolved by *Candida rugosa* Lipase catalyzed kinetic resolution[3]. The optical purity of product was checked by GC/MS (see experimental procedures).

Compounds were tested, as described in Chapter 2, by Jianghai Ho in the Matsunami lab at Duke University. 2,2-dimethyloctanal was unable to activate OR-I7. All three 2-methyloctanal samples were more active than 2,2-dimethyloctanal but much less active than octanal. This trend of decreasing activity with more steric bulk supports that there is steric constraint near the aldehyde group. Among the three 2-methyloctanal samples, the S isomer showed a slightly higher activity than the R isomer, but the difference wasn't significant. Overall, although OR-I7 may be able to weakly distinguish different conformation at C_2, the major effect of C_2 substitution is likely to be steric effect that prevents the activation of OR-I7.

In Chapter 3, the panel screening was done on different rat OSNs, and we didn't know how OR-I7 responds to the 2,2-difluorooctanal and other control compounds. (Those experiments were done on cells whose expressed receptor was not identified.) This result (Fig. 4.3) showed the pattern of responses supporting that OR-I7 is one of the gem-diol receptors. 2,2-difluorooctanal that exist almost completely in the gem-diol form showed much higher potency than octanal. 2,2-dimethyloctanal inactivity can be affected by electron effect (favors carbonyl form) or steric effect (too bulky). Since the methyls are only slightly larger than the fluorines, we tentatively conclude that the electron effect plays more important role for the 2-methyl compounds.

An interesting phenomenon was observed on 2-methyloctanal. Although aldehydes are known to be unstable to autoxidation by air, the 2-methyloctanal was found much more labile to

air oxidation than other "normal" aldehydes that were used. Without careful protection and storage, it can be oxidized within few days. This phenomenon seemed to be specific to C_2 mono substituted aldehyde. Other 2-alkyl substituted aldehydes such as compound **1.6** (Chapter 1), compound **2.17** and cyclopentylmethanal (Chapter 2) all showed to be very unstable to air oxidation. The electron donating methyl group increases the electron density on the carbonyl carbon making the aldehyde easier to oxidize. Meanwhile, the methyl group adds steric hindrance for the oxygen to approach the carbonyl carbon, for example, two methyl groups will greatly block the approaching of oxygen and offset the electron effect. With one methyl group at C_2, oxygen can still access the carbonyl carbon easily and react with the more reactive aldehyde group.

In contrast, difluorooctanal (Chapter 3) showed to resist air oxidation (Prof. Ryan suggested further studies of checking if difluoroaldehyde even resist bleach as fragrances that resist bleach are not numerous and could be of value to the fragrance industry). We tried to characterize the stability of aldehydes towards oxidation by measuring their oxidation potential using cyclic voltammetry. However, with the equipment and setup available to us and CCNY chemistry professor Ronald Birke, who tried to collaborate with us, we weren't able to obtain the oxidation potential.

Hydrogen bonding ability of aldehyde group is required for binding to OR-I7

It has been predicted by computation with rhodopsin based model that the aldehyde group form hydrogen bond with a protonated amino acid residue in the OR-I7[4]. To test whether this hydrogen bond is required for the recognition, fluorinated hydrocarbons were designed and as octanal analogues. Compound **4.4** and **4.5** keep approximate same length as octanal with 8

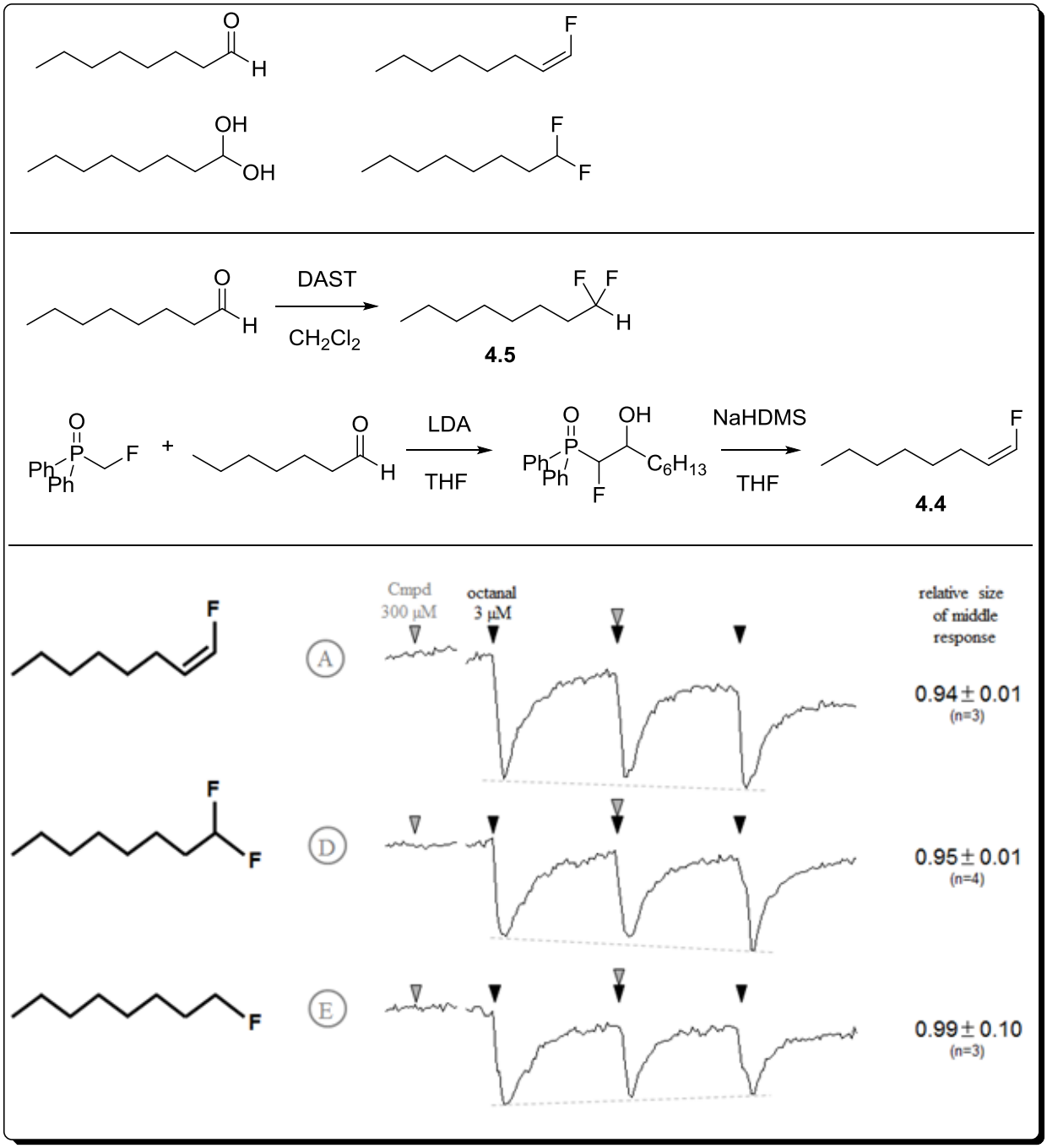


Figure 4.4 Fluorinated octanal analogues are neither agonists nor antagonist of OR-I7

carbons chain backbone. Fluorine atom, having close Van der Waals radius as oxygen (F: 1.47 Å, O: 1.52 Å) while not acting as hydrogen bond acceptor on a C-F bond[5], was used to replace oxygen. 1-fluorooctene (compound **4.4**) mimics the planar shape of carbonyl group with the sp^2

C1 carbon. 1,1-difluorooctane (compound **4.5**) mimics the gem-diol form. The synthesis of compound 4.4 and 4.5 are shown in Figure 4.4.

The result showed that compound **4.4** and **4.5** were neither agonists nor antagonists (Figure 4.2), which means they don't bind to OR-I7. As discussed in Chapter 3, some octanal responding ORs recognize octanal by its gem-diol form. OR-I7 appears to be one of those gem-diol receptors (Figure 4.3). The result that both fluorinated analogues don't bind to OR-I7 supports that hydrogen bonding of aldehyde is critical for effectively binding to OR-I7.

Why ketones are not recognized by ORI7?

2-octanone has been tested and found inactive for OR-I7. The extra methyl group on the carbonyl group, in place of the aldehyde proton, is likely a steric hindrance to prevent interactions of the carbonyl group/gem-diol to the receptor. Meanwhile, another reason for 2-octanone not able to activate gem-diol receptor OR-I7 could be that ketone is less prone to form gem-diol hydrate than aldehyde. In water propanal (K_{hyd}=0.83) forms ~45% hydrate at equilibrium, but acetone ($K_{hyd}=2*10^{-3}$) forms only ~0.3% hydrate (Figure 4.5a). Electron withdrawing fluorine at C_1 can upset keto/hydrate equilibrium and lead to a much higher hydration. 1,1,1-Trifluoroacetone (K_{hyd}=35) exists mostly in the gem-diol form (~97%, Figure 4.5a). We asked if the hydration be able to overcome the disadvantage of the steric hindrance, and makes the ketone (in gem-diol form) an agonist of OR-I7? Compound **4.7** and **4.8** were synthesized (Figure 4.5b) and tested to answer this question.

The result was surprising that 2-octanone and **4.7** both appear to be week antagonists and have approximately equal potencies (Figure 4.5c). We interpret that the result is due to the relative stability of the normal and fluorinated 2-octanone. The fluorinated analogues, e.g. **4.7**

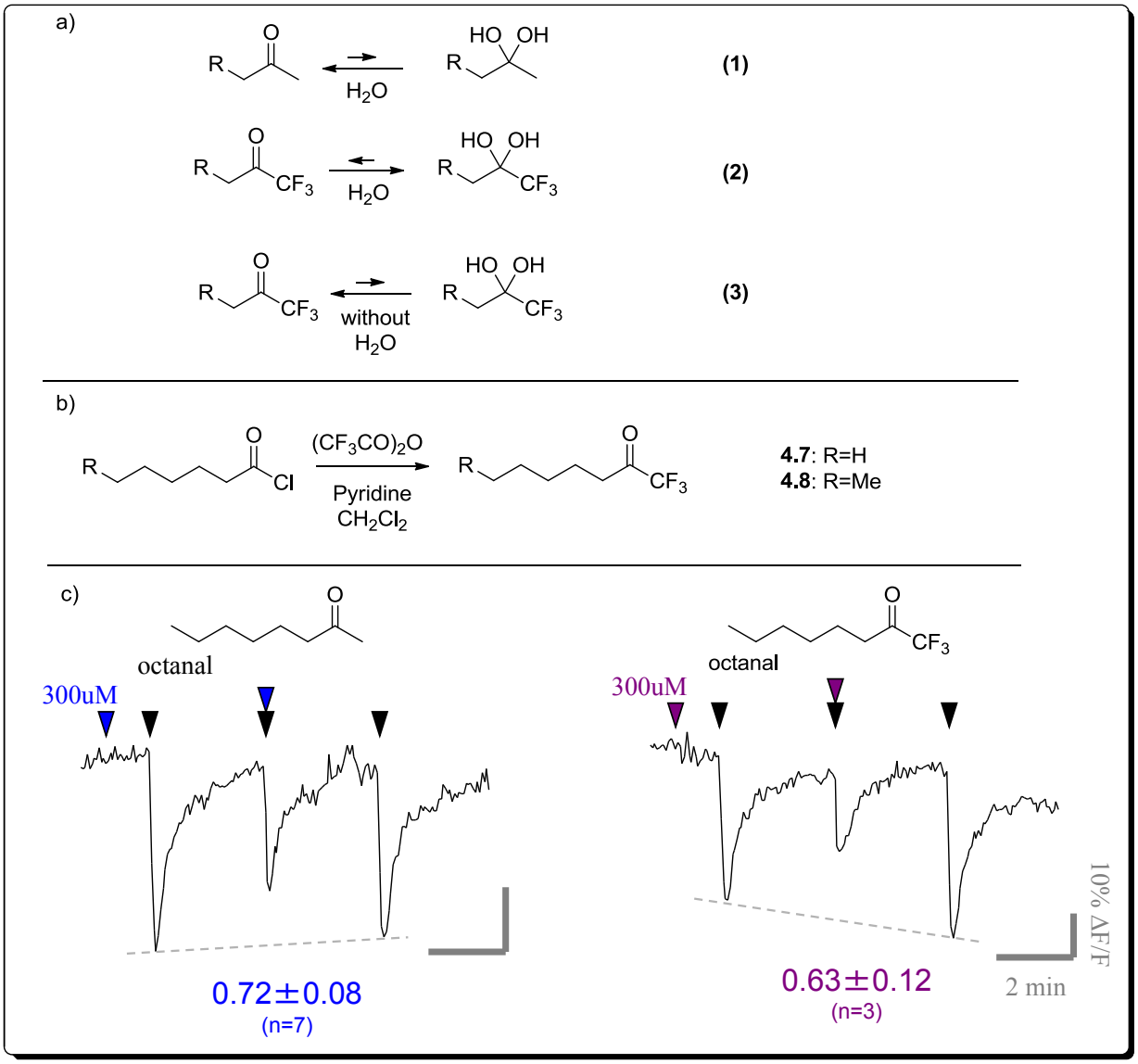


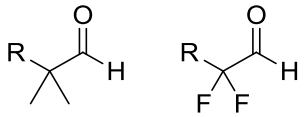
Figure 4.5 2-Octananone and trifluoro-2-octanone weekly antagonize octanal activation of rat OR-I7 (octanal concentration, 3 μM).

and difluorooctanal, form mostly hydrates in aqueous solution. They have to leave the aqueous phase and enter a hydrophobic environment to bind to the receptor. For 2,2-difourooctanal, once the hydrate is formed, it's stable even when removed from aqueous environment. After column chromatography and longtime storage the hydrate is still visible/detectable by TLC and NMR. In contrast, no hydrate of **4.7** was seen by TLC or NMR, which suggests that the gem-diol form of **4.7** may be not stable outside aqueous solution and dehydrates quickly without aqueous

environment (Figure 4.5a, equation 3). Therefore **4.7** may enter the hydrophobic transmembrane domain in its keto form and act in the same way as 2-octanone.

2,2-difluoroaldehydes and 2,2-dimethylaldehydes

Besides 2,2-difluorooctanal and 2,2-dimethyloctanal (Chapter 3), a series of 2,2-difluoroaldehydes and 2,2-dimethylaldehydes from C5 to C10 were synthesized (see supporting information in Chapter 3). The 2,2-difluoroaldehyde, 2,2-dimethylaldehyde and *n*-aldehyde with the same number of carbons were to tested by panel screen (Chapter 3). The goal was to seek a general trend of how much aldehyde activating odorant receptors detect the aldehyde group by its gem-diol form. Unfortunately, the solution-based screen on C6 series (hexanal, 2,2-difluorohexanal and 2,2-dimethylhexanal) gave too little hexanal activating cells as a starting population. We think this is due to increased water solubility of the smaller aldehyde and the fact that we test aqueous solution of the compounds, rather than the in vivo method of gas-mucus transfer. In addition, the smaller difluoro analogs were so volatile that they could not be separated from methylene chloride by distillation. We therefore focused on the 8-carbon series in Chapter 3, and the rest of the compounds were not tested.



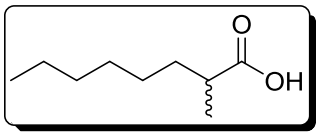
R= n-C_3H_7, n-C_4H_9, n-C_5H_{11}, n-C_7H_{15}, n-C_8H_{17}

Figure 4.6 2,2-difluoroaldehydes and 2,2-dimethylaldehydes with different chain length

4.3 Experimental Procedures

NMR spectra were recorded on a Varian Mercury-300 spectrometer. Chemical shifts are reported in parts per million (ppm) referenced to the appropriate solvent peak. Infrared (IR)

spectra were recorded using a Thermo Nicolet 6700 FT-IR spectrometer, and are reported in wavenumbers (cm^{-1}). GC/MS analyses were obtained on Shimadzu GC/MS QP5000 with GC-17A Gas Chromatograph (capillary column: DB-1-30N-STD). Temperature programs for GC analysis: Program 1: Injection temperature: 280 °C; held at 60 °C for 1 min, heated from 60 to 200 °C at 5 °C/min, and held at 200 °C for 4 min; Program 2: Injection temperature: 280 °C; held at 60 °C for 1 min, heated from 60 to 280 °C at 10 °C/min, and held at 280 °C for 3 min; Program 3: Injection temperature: 200 °C; held at 50 °C for 1 min, heated from 50 to 150 °C at 4 °C/min, and held at 150 °C for 4 min; Program 4: Injection temperature: 200 °C; held at 60 °C for 1 min, heated from 60 to 150 °C at 4 °C/min, and held at 150 °C for 2 min



rac-2-methyloctanoic acid (4.9)[6]:

Sodium (2.28 g, 0.099 mol) was added in small pieces to absolute ethanol (61 mL) under nitrogen. After all the Na had reacted, diethyl 2-methylmalonate (17.78 mL, 0.104 mol) was added over 10 min. The mixture was refluxed for 5 min and 1-bromohexane (15.33 g, 0.093 mol) was added over 8 min. The mixture was refluxed for 2 h and then neutralized with 1 drop of acetic acid. About 3/4 of ethanol was evaporated and H_2O (80 mL) was added. The organic layer was separated and the aqueous layer was extracted with ether. The combined organic layers were washed with brine, dried ($MgSO_4$) and concentrated to give 26 g oil. It was hydrolyzed by refluxing with KOH (20.9 g, 0.373 mol) in 95% EtOH (160 mL) for 4 h. About 130 mL EtOH was removed and 160 mL H_2O was added. The mixture was extracted with ether. The aqueous layer was acidified to pH=1 at 0 °C with 6 M HCl. The organic layer was separated and the aqueous layer was extracted with ether. The combined organic layers were washed with H_2O, brine, dried and evaporated to dryness. The resulting solid was recrystallized from heptane to give 15 g white crystal diacid. The diacid was heated to 190 °C for 4 h. The

liquid obtained was distilled under vacuum to give 11 g to give colorless liquid **4.9** (75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.43 (h, *J* = 7.0 Hz, 1H), 1.65 (dt, *J* = 14.5, 7.2 Hz, 1H), 1.47–1.22 (m, 10H), 1.15 (d, *J* = 7.0 Hz, 3H), 0.91–0.80 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 183.83, 39.62, 33.75, 31.89, 29.39, 27.32, 22.83, 17.04, 14.29. IR (neat), ν: 2929, 2859, 2656, 2567, 1701, 1467, 1417, 1378, 1291, 1237, 942; GC retention time: 13.97 min (method: program 1), MS (EI): 158 (M^+).

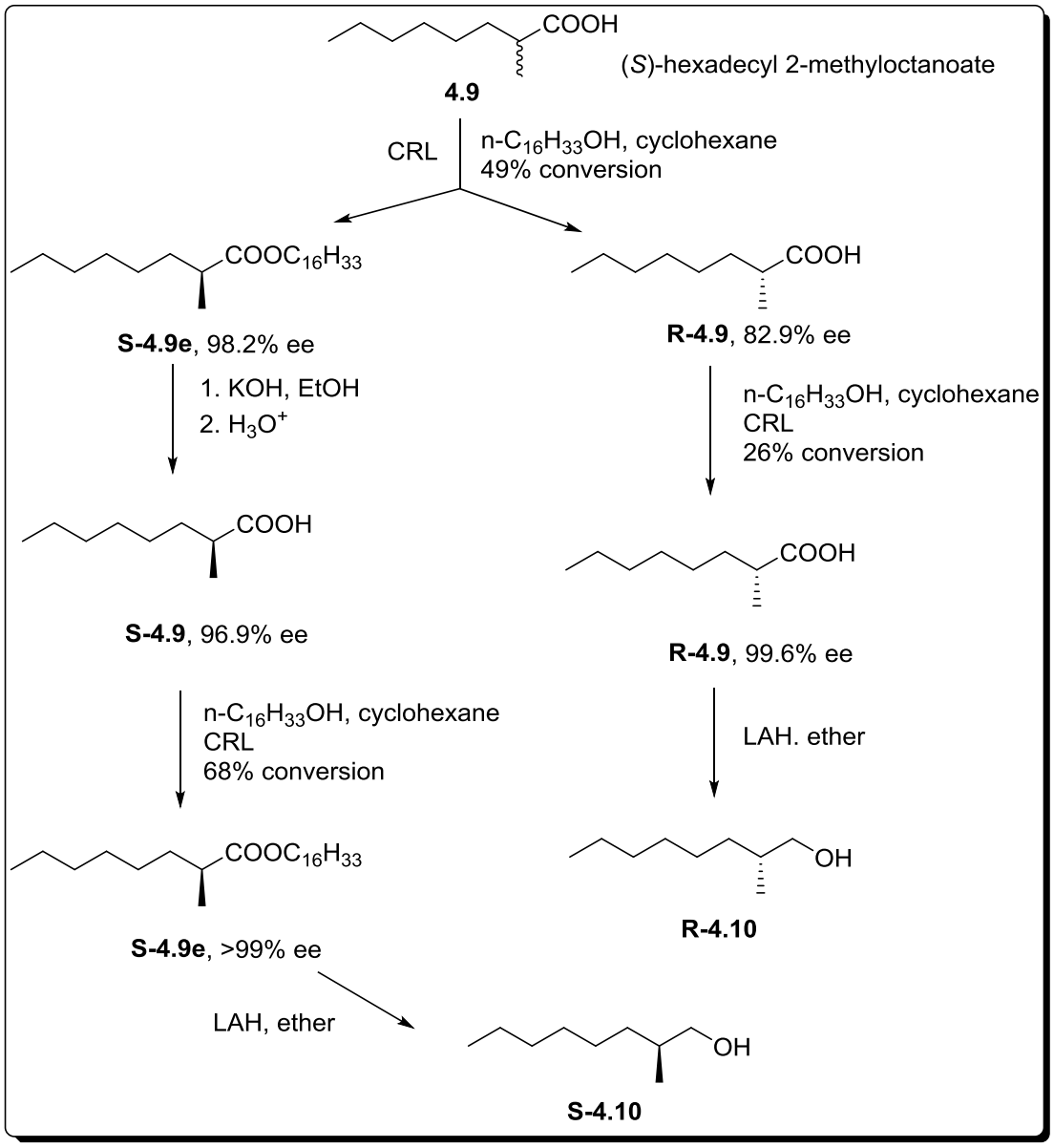
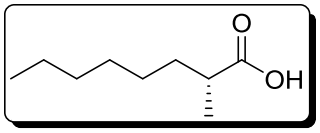
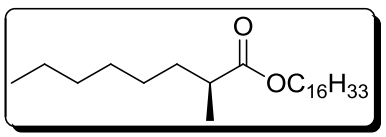


Figure 4.7 Resolution of 2-methyloctanoic acid



(R)-2-methyloctanoic acid (R-4.9)[3, 6-7]:

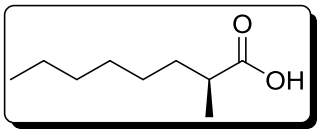
A solution of rac-2-methyloctanoic acid (4 g, 25.3 mmol), 1-hexadecanol (4.6 g 19.0 mmol), octadecane (internal standard, 1.03 g), Na_2SO_4 (4.8 g), $Na_2SO_4 \cdot 10H_2O$ (5.45 g) in cyclohexane (170 mL) was stirred for 15 min. *Candida rugosa* lipase (CRL) (2.86 g) was added. The mixture was stirred at room temperature and monitored by GC/MS. The reaction was stopped at 49% conversion after 4 h by filtering through celite and washed with 50% cyclohexane and 50% pentane. The combined organic layers were diluted with pentane, extracted with 10% Na_2CO_3 and brine. The aqueous layer was acidified to pH=1 with 6 M HCl and extracted with ether. The ether extract was washed with brine dried ($MgSO_4$) and evaporated to give 2.056 g R-acid which contained small amount of 1-hexadecanol. A second acid-base extraction gave 2.010 g (R)-2-methyloctanoic acid (50.3%, yield, 82.9% ee). A second lipase catalyzed esterification of this acid (1.8 g, 11.4 mmol) using lipase (5.13 g) and 6 equivalent 1-hexadecanol (16.5 g, 68.3 mmol) was stopped at 26% conversion after 29 h. The product was purified as described above to give 1.33 g colorless liquid **R-4.9** (74% yield, 99.6% ee). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.43 (h, J = 7.0 Hz, 1H), 1.65 (dt, J = 14.5, 7.2 Hz, 1H), 1.47–1.22 (m, 10H), 1.15 (d, J = 7.0 Hz, 3H), 0.91–0.80 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 183.89, 39.63, 33.75, 31.89, 29.39, 27.32, 22.83, 17.04, 14.30; IR (neat), ν: 2929, 2859, 2659, 2569, 1701, 1467, 1417, 1378, 1291, 1237, 942, 725; GC retention time: 14.55 (method: program 1); MS (EI): 158 (M^+).



(S)-hexadecyl 2-methyloctanoate (S-4.9e):

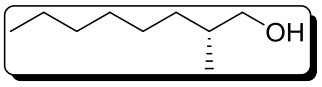
From the reaction described above, CRL catalyzed esterification of rac-2-methyloctanoic acid (4 g, 25.3 mmol) with 1-hexadecanol (4.6 g, 19.0 mmol) was

stopped at 49% conversation. The organic layers obtained after extracting with 10% Na_2CO_3 were dried ($MgSO_4$) and concentrated to give an oil containing 1-hexadecanol, octadecane, and (S)-hexadecyl 2-methyloctanoate. The mixture was chromatographed on silica gel with hexane then 10% ether 90% hexane to give 4.235 g color less liquid (S)-2-methyloctanoate (44% yield, 98.2% ee). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.03 (td, J = 6.6, 1.1 Hz, 2H), 2.43–2.32 (m, 1H), 1.59 (dq, J = 13.6, 6.5 Hz, 4H), 1.23 (s, 34H), 1.17–1.05 (m, 3H), 0.91–0.80 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.30, 64.50, 39.85, 34.09, 32.15, 31.94, 29.92, 29.89, 29.81, 29.77, 29.60, 29.48, 29.43, 28.88, 27.43, 26.16, 22.93, 22.83, 17.36, 14.36; IR (neat), ν: 2923, 2853, 1736, 1465, 1169, 1147; GC retention time: 23.31 min (method: program 1).



(S)-2-methyloctanoic acid (S-4.9)[7]:

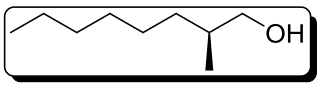
(S)-hexadecyl 2-methyloctanoate was stirred with KOH in ethanol for 5 h. The mixture was diluted with H_2O and acidified with 6 M HCl. The solution was extracted with ether. The organic layer was extracted with 10% Na_2CO_3 solution. The Na_2CO_3 extract was acidified to pH=1 with 6 M HCl and extracted with ether. The ether layer was washed with brine and dried. Evaporation of ether gave 1g colorless liquid (S)-2-methyloctanoic acid (58%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.43 (h, J = 7.0 Hz, 1H), 1.65 (dt, J = 14.5, 7.2 Hz, 1H), 1.47–1.22 (m, 10H), 1.15 (d, J = 7.0 Hz, 3H), 0.91–0.80 (m, 3H).



(R)-2-methyloctanol (R-4.10)[8]: Procedure for LAH reduction

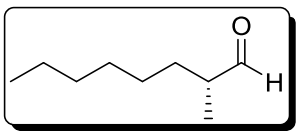
To a mixture of LAH (0.43 g, 11.4 mmol) and 12 mL dry ether cooled to 0°C, was added **R-4.9** (1.2 g, 7.58 mmol) in 6 mL dry ether dropwise with stirring. The mixture was stirred at room temperature for 2 h and cooled to 0°C again. The mixture was worked up by dropwise and sequential addition of 0.43 mL H_2O, 0.43 mL 15% NaOH solution, 1.29 mL H_2O and then

stirred for 5 min. The mixture was filtered and washed with ether. The resulting solution was dried with $MgSO_4$ and solvent was evaporated to give 1.07 g (98%) colorless liquid **R-4.10**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.41 (ddd, *J* = 30.4, 10.4, 6.3 Hz, 2H), 1.65–1.48 (m, 1H), 1.43–1.20 (m, 10H), 1.13–0.97 (m, 1H), 0.94–0.74 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 68.54, 35.93, 33.34, 32.06, 29.81, 27.14, 22.86, 16.77, 14.30: IR (neat), ν: 3332 (br), 2925, 2858, 1466, 1378, 1035, 724; GC retention time: 8.19 min (method: program 2); MS (EI): 126 ($M-H_2O^+$).



(S)-2-methyloctanol (S-4.10)[8]:

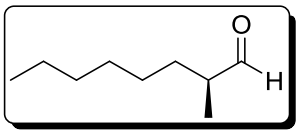
A second lipase catalyzed esterification of (S)-2-methyloctanoic acid (0.95 g, 6 mmol) with lipase (0.676 g) and 1-hexadecanol (1.09 g, 4.5 mmol) was stopped at 68% conversion after 5 h. The product was purified as described above to give 1.556 g (S)-hexadecyl 2-methyloctanoate (68% yield, >99% ee). This (S)-hexadecyl 2-methyloctanoate was reduced by LAH (see above) to give a mixture of (S)-2-methyloctanol and 1-hexadecanol. The mixture was fractionally distilled under vacuum to give 0.46 g colorless liquid (S)-2-methyloctanol (53%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.55–3.31 (m, 2H), 1.67–1.49 (m, 1H), 1.47–1.13 (m, 10H), 1.06 (dd, *J* = 16.9, 7.4 Hz, 1H), 0.95–0.75 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 68.61, 35.96, 33.34, 32.07, 29.82, 27.14, 22.88, 16.78, 14.32; IR (neat), ν: 3333, 2925, 2858, 1466, 1378, 1035, 724; GC retention time: 8.15 min (method: program 2); MS (EI): 126 ($M-H_2O^+$)



(R)-2-methyloctanal (R-4.3)[9]:

A solution of **R-4.10** (0.4 g, 2.78 mmol) in dry CH_2Cl_2 (5 mL) was added to PCC (0.9 g, 4.17 mmol) and silica gel (0.9 g) in dry CH_2Cl_2 (10 mL) with stirring. After stirring for 3 h, the mixture was filtered through a silica gel pad and washed with excess CH_2Cl_2. The yellow liquid from evaporation of CH_2Cl_2 was chromatographed on silica gel with 5% ether

and 95% hexanes to give 0.3 g (75%) colorless liquid **R-4.3**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.58 (d, *J* = 2.0 Hz, 1H), 2.29 (td, *J* = 6.8, 2.0 Hz, 1H), 1.75–1.61 (m, 1H), 1.41–1.15 (m, 9H), 1.06 (d, *J* = 7.0 Hz, 3H), 0.95–0.79 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 205.73, 46.54, 31.86, 30.71, 29.50, 27.11, 22.80, 14.28, 13.51; IR (neat), ν: 2958, 2927, 2857, 2703, 1727, 1459, 1377, 925; GC retention time: 7.15 min (method: program 2); MS (EI): 142 (M^+)



(S)-2-methyloctanal (S-4.2)[10]:

PCC oxidation (see above) of **S-4.10** to give colorless liquid **S-4.2**. Yield: 67%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.58 (d, *J* = 2.0 Hz, 1H), 2.29 (td, *J* = 6.8, 2.0 Hz, 1H), 1.75–1.61 (m, 1H), 1.32–1.22 (m, 9H), 1.06 (d, *J* = 7.0 Hz, 3H), 0.91–0.79 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 205.75, 46.54, 31.86, 30.70, 29.50, 27.11, 22.80, 14.29, 13.51; IR (neat), ν: 2929, 2858, 2703, 1728, 1464, 1379, 926; GC retention time: 7.11 min (method: program 2); MS (EI): 142 (M^+).

Determination of enantiomeric excess (ee):

Ester or aldehyde (0.2 mmol) in 0.5 mL dry ether was added to 20 mg $LiAlH_4$ in 1 mL ether. The mixture was stirred for 1 h, and then quenched with 20 μL H_2O, 20 μL 15% NaOH and 60 μL H_2O. After stirred briefly, the mixture was filtered, dried and solvent was evaporated to give

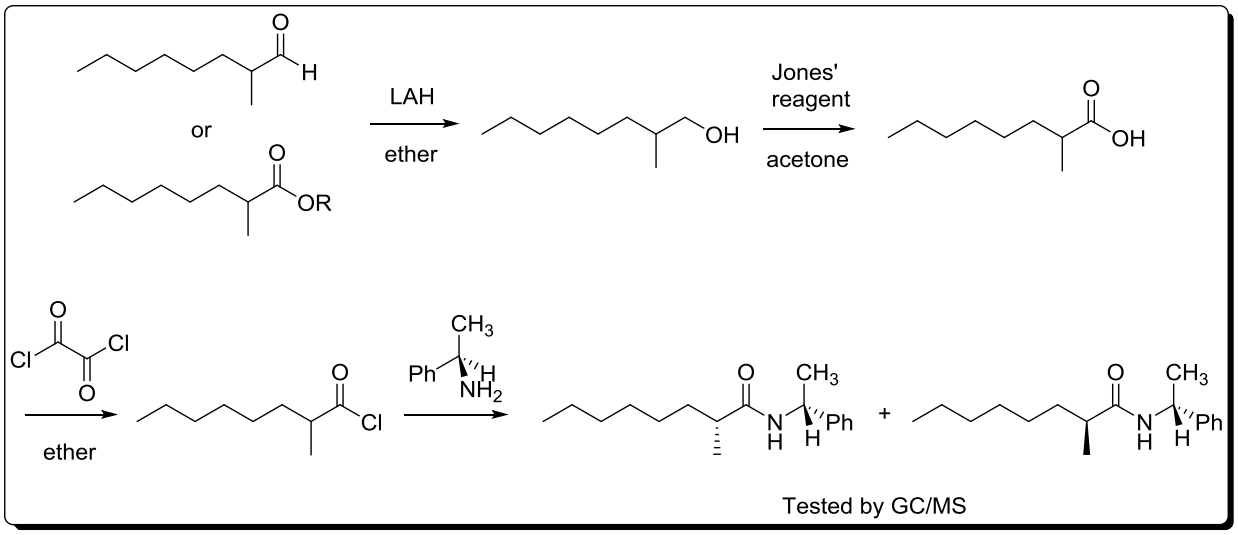
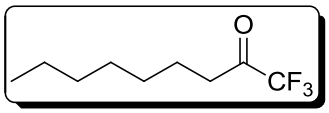


Figure 4.8 Diastereomeric amide preparation for determine ee%

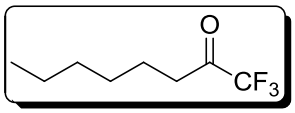
corresponding alcohol. The alcohol was dissolved in 1 mL acetone and cooled to 5 °C. 50 µL 2.67 M Jones' reagent was added. The mixture was stirred for a few minutes and filtered through celite which was washed with ether and pentane. The combined organic layers were extracted with 10% Na_2CO_3 (1 mL X 2). The Na_2CO_3 extract was acidified with 6 M HCl and extracted with ether (1 mL X 2). The combined ether layer was dried and concentrated to give corresponding acid. To a solution of 10 µL of acid in 1 mL dry ether, was added 12 µL oxalyl chloride and 10 µL DMF. After 10 min, 20 µL D(+)-α-methylbenzylamine (99+% pure, 99% ee from Acros) was added. The resulting mixture was diluted with ether (0.5 mL), washed with 1 mL H_2O, 1 mL 10% Na_2CO_3, 1 mL brine and dried. The ratio of the diastereomers in the organic layer were checked quantitated by GC/MS.



1,1,1-trifluorononan-2-one (4.8)[11]

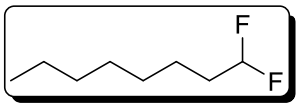
To a solution of trifluoroacetic acid (14.66 mL, 0.10 mol) in anhydrous CH_2Cl_2 (150 mL) was added octanoyl chloride (prepared from octanoic acid and oxalyl chloride, 2.81 g, 17.3 mmol). The mixture was cooled to 0 ºC and pyridine (11.15 mL,

0.14 mol) was added. The mixture was stirred at room temperature for 1 h. Water (20 mL) was added dropwise to the mixture until the evolution of CO_2 ceased. The reaction mixture was then poured into water (300 mL) and extracted with CH_2Cl_2. The organic layers were dried with $MgSO_4$. CH_2Cl_2 was removed by simple distillation. The residue was purified by column chromatography (15% CH_2Cl_2, 85 pentane) to give 1.1 g (33%) colorless liquid **4.8**. ^{1}H NMR (300MHz ,$CDCl_3$) δ 2.71 (t, *J* = 7.2 Hz, 2 H), 1.68 (t, *J* = 7.2 Hz, 2 H), 1.30 (m, 8 H), 0.88 (t, *J* = 6.9 Hz, 3 H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 36.4, 31.5, 28.9, 28.7, 22.6, 22.4, 14.0; ^{19}F NMR (282MHz ,$CFCl_3$) δ = -79.8 (s); IR (neat), ν: 2959, 2932, 2860, 1766, 1488, 1406, 1290, 1282, 1209, 1151, 1051, 1003, 709; GC retention time: 8.32 min (method: program 3); MS (EI): 127 ($M\text{-}CF_3^+$).



1,1,1-trifluorooctan-2-one (4.7)

Prepared from heptyl chloride by the same procedure as **4.8**. ^{1}H NMR (300MHz, $CDCl_3$) δ 2.72 (t, *J* = 7.3 Hz, 2 H), 1.67 (m, 2 H), 1.30 (s, 6 H), 0.87 (t, *J* = 7.3 Hz, 3 H); ^{13}C NMR (75MHz ,$CDCl_3$) δ 36.4, 31.3, 28.4, 22.4, 14.0; IR (neat), ν: 2961, 2934, 2863, 1765, 1205, 1147, 1054, 991; GC retention time: 5.76 min (method: program 3); MS (EI): 113 ($M\text{-}CF_3^+$).



1,1-difluorooctane (4.5)

To an ice-cold solution of octanal (1.45 mL, 9.3 mmol) in CCl_4 (6 mL) under N_2, was added DAST (2.3 mL, 17.4 mmol). The mixture was stirred at 0 ºC for 90 min and at room temperature for 18 h. The mixture was then added dropwise with stirring to ice water, neutralized with Na_2CO_3 and extracted with pentane. The extract was washed with water and brine, dried with $MgSO_4$ and concentrated by distillation. The crude product was purified by column

chromatography (1% ether, 99% pentane) to give 0.8 g (57%) product. ^{1}H NMR (300MHz ,$CDCl_3$) δ 5.78 (tt, *J* = 4.7, 47.8 Hz, 1 H), 1.95 - 1.70 (m, 2 H), 1.52 - 1.15 (m, 10 H), 0.88 (t, *J* = 6.7 Hz, 3 H); GC retention time: 5.20 min (method: program 4); MS (EI): 150 (M^+).

References

Introduction

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