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ARTICLE

Ionic Conjugates of Lidocaine and Sweeteners as Better Tasting Local Anesthetics for Dentistry

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Iryna O. Lebedyeva,[a,b] Alexander A. Oliferenko,[b,c*] Polina V. Oliferenko,[c] Robert A. Hromas,[d] John Neubert,[e] Robert M. Caudle,[f] Jay Wickersham,[f] William L. Castleman,[g] Gary I. Altschuler,[h] David A. Ostrov,[i] C. Dennis Hall[b] and the late Alan R. Katritzky[b]

Lidocaine is the most widely utilized intraoral injected dental anesthetic, used for more than 500 million dental injections per year. Local anesthesia is essential for pain-free dentistry, yet intraoral injections are often considered painful and a source of anxiety for many patients. Any new anesthetics that will reduce the stress and anxiety of dental injection are expected to be beneficial. A novel chemical approach to taste modulation is proposed, in which the lidocaine cation is coupled with anionic sweeteners such as saccarinate and acesulfamate. The ionic conjugates synthesized using anion exchange techniques, were much less bitter, demonstrated a high local anesthetic potential in animal studies, and were as safe as the original hydrochloride. Based on the currently robust market for lidocaine it is expected that the resulting anesthetics will be in high demand in clinical practices worldwide.

Introduction

Local anesthetics (LA) are widely used in oral and gastrointestinal health care practices either topically or as injections. Intraoral local anesthesia is essential for delivering dental care. However, it is often perceived by patients as the most painful and in some instances as the only objectionable part of the treatment, leading in extreme cases to avoidance of dental care. A significant number of patients detect an unpleasant metallic taste following intraoral injections of lidocaine which adds to the patient's source of anxiety. Most local anesthetics belong to two chemical classes: amino amides (e.g. lidocaine, mepivacaine, ropivacaine, articaine) and amino esters (e.g. benzocaine, procaine, chloroprocaine), shown in Figure 1.

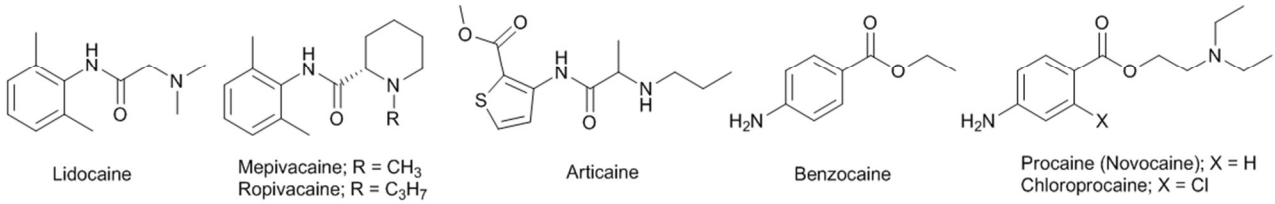


Fig. 1 Some local anesthetics used in dental and gastrointestinal practices.

Clinical LAs have amphipathic nature, *i.e.* comprise lipophilic and hydrophilic properties: the presence of lipophilic aromatic groups (benzene, thiophene rings) facilitates membrane permeability, whereas hydrophilic carboxylic, amide, and protonated amino groups enable water solubility of the drug molecule.[1] In their pure form, LAs are weak bases poorly soluble in water and unstable in open air. Consequently, in topical and injection formulations, LAs are neutralized with acids to form LA salts, which are water soluble and comparatively chemically stable.[1]

Taste is an important factor in the development of oral dosage LAs. Bitter taste is common for natural alkaloids (quinine, nicotine, caffeine, papaverine, *etc*.), some peptides and hydrophobic amino acids,[2] as well as many synthetic compounds. A disagreeable taste of pharmaceutical compounds is generally not of concern for pelleted drugs, since the tastes are masked by an exterior coating. In the case of liquid, aerosol, or chewable forms, an unpleasant taste of the active ingredient can become a dominating factor governing the patients' acceptance of the drug. The importance of patient-friendly dosage forms of bitter drugs, especially for pediatric, geriatric, and bedridden patients encourages pharmaceutical manufacturers to develop various taste-masking methods.[3] The simplest way to obscure an unpalatable taste is to include sweeteners and flavors into a formulation. For example, the bitter taste of docusate (common laxative ingredient) was masked with sorbitol, xylitol, and sucralose coating.[4] Sucrose and sorbitol

are added to mask the bitter after taste of liquid ibuprofen suspension[5] and docusate compositions[4] used for oral administration. Another way to reduce bitter taste is by adding bitterness inhibitors, of which adenosine mono-, di-, and triphosphate, thymidine monophosphate, adenosine succinate, inosinic acid,[6] sodium glutamate, sodium gluconate, L-lysine, and L-arginine amino acids[7] are a few notable examples. Yet another group of masking techniques is based on chemical and physical modification methods and includes microencapsulation, ion exchange, and complexation methods. For example, Makino *et al*. proposed to mitigate the taste of a cocktail composed of allopurinol and local anesthetics by loading the mixture in microspheres composed of carrageenan.[8]

Lidocaine hydrochloride is commonly used for local analgesia prior to performing painful medical procedures. In dentistry, it was found to provide effective pain control during scaling, root planning in treatment of periodontitis,[12] and to stabilize vital signs of patients under general anesthesia during dental surgery and rehabilitation.[13] Lidocaine hydrochloride is used for pharyngeal anesthesia before upper gastrointestinal tract endoscopy,[14] and was shown to improve patients comfort during extensive nasal surgery.[15] Numerous attempts have been described in the academic and patent literature to neutralize its bitter taste by formulating lidocaine hydrochloride with natural or artificial sweeteners.[16,17]

What makes lidocaine hydrochloride so bitter? Chemically, lidocaine hydrochloride is a salt of an arylalkyl amine. Natural alkaloids such as caffeine and many others have similar structures and are known to be bitter in taste. An additional factor in lidocaine is the chloride anion, which is well known for inducing saltiness or bitterness in many inorganic salts. Thus a natural solution to improve the taste of lidocaine salts would be to exchange chloride for some better-tasting anions. An idea to combine biologically active ions to design dual-functional pharmaceutical compounds was formulated by Rogers *et al*. in terms of the ionic liquids concept.[18,19] Rogers *et al*. proposed to pair anions and cations with specified activities, including anti-bacterial, anti-inflammatory, anesthetic, emollient, *etc*. to optimize chemical (solubility), biological (bioavailability, pharmacokinetics), and physical (thermal stability) of novel ionic liquid pharmaceuticals.[20,21]

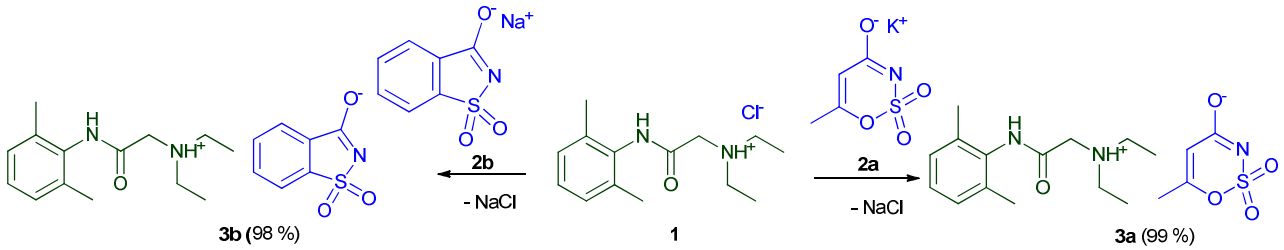
Several commercially available artificial sweeteners currently exist in the anionic form[22] and can be readily incorporated into ionic compounds with lidocaine using ionic liquid methodology. For example, saccharinate was selected as counter anion in ciprofloxacin saccharinate and other fluoroquinolone antimicrobials because of its sweet taste, improved aqueous solubility, and ability to form salts and co-crystals.[23] Substitution of conventional chloride anion by saccharinate in tramadol hydrochloride improved the release profile and stability of the active ingredient.[24] It was also demonstrated that saccharinate as well as acesulfamate, possess low toxicity.[25] Another well-known option to mitigate bitterness is to introduce gluconate anion, which is not a sweetener *per se*, but is used in various injectable pharmaceutical formulations, including chlorohexidine gluconate,[26] calcium gluconate,[27] ferric gluconate,[28] and antimony gluconate.[29]

The aim of this work is thus to explore the combination of sweetener anions with lidocaine and other cationic local anesthetics and to test the resultant salts for anesthetic activity, mechanism of action, and safety. Development of such novel formulations can provide well-tasting water-soluble oral gels and injections for use in dentistry, otolaryngology, and endoscopy of the upper gastrointestinal tract. The non-stinging effect of injections of the novel lidocaine salts can expand the range of product use to the area of general local anesthetic injections and procedures.

Results and discussion

Anion Exchange with Commercially Available Sweeteners

Synthesis of lidocaine acelsulfamate **3a** and acyl saccharinate **3b** was carried out by stirring equimolar quantities of lidocaine hydrochloride **1** and potassium acesulfamate **2a** or sodium saccharinate **2b** in acetonitrile in ultrasonic bath at 50 ^{o}C for 5 h. Sodium chloride was then separated by filtration through a 0.22 micron membrane filter. After evaporation of solvent, the products were isolated in 98 % for **3a** and 99 % for **3b** yields. Salts **3a** and **3b** are ionic liquids (IL) in the form of thick oils and are readily soluble in organic solvents and water.



Scheme 1 Synthesis of lidocaine acesulfamate **3a** and lidocaine saccharinate **3b**.

The ^{1}H NMR of **3a** showed a broad singlet in the aromatic region at 7.12 ppm, a singlet for one proton at 5.54 ppm, and a singlet for 2 protons at 4.29 ppm. Signals for the methyl group of saccharinate and the ethyl group of the lidocaine moiety were located between 3.35-1.32 ppm. The ^{13}C spectrum of **3a** had three signals at 172.6, 165.0, and 163.9 ppm, four signals of the aromatic carbons of lidocaine between 136.8-128.9, one signal at 102.4 and five signals of aliphatic carbons of both ions of **3a** between 54.4-9.6 ppm.

The ^{1}H spectrum of **3b** revealed signals of seven aromatic protons between 7.75-7.09 ppm, a CH_2 group singlet at 4.26 ppm, and a singlet for two Ar-CH_3 groups of lidocaine at 2.20 ppm. The ethyl group of lidocaine was observed as a quartet of two CH_2 groups at 3.32 ppm and a triplet of two CH_3 groups at 1.35 ppm. The ^{13}C spectrum of **3b** contained two signals in the downfield at 170.2 and 163.6 ppm (NC=O), ten signals of the aromatic carbons between 143.8-

120.8 ppm and four signals of aliphatic carbons between 53.0-9.0 ppm.

Lidocaine gluconate **3c** was synthesized according to a previously reported protocol.[30] A 20 % solution of **2c** was heated under reflux and an equimolar amount of lidocaine free base **4** (Scheme 2) in EtOH was added in 1 mL aliquots to the solution. The pH of the solution changed from 2 (before addition of lidocaine) to 7, after 7 mL of lidocaine EtOH solution had been added. The pH changed back to 6 during 5 h of stirring the mixture, and when the pH dropped to 6, more lidocaine solution was added. Gluconic acid **2c** coexists in aqueous solutions with the respective lactone, glucono delta-lactone and upon adding a base, the lactone hydrolizes and gluconic acid becomes more available. The reaction took almost 24 h for all the gluconic acid to react and pH=7 was achieved. The reaction mixture was then evaporated using azeotropic distillation first with toluene and then with absolute ethanol.

Scheme 2 Synthesis of lidocaine gluconate **3c**.

According to ^{1}H and ^{13}C NMR data, the purity of the **3c** was 95 %, with residual water making up the balance. The ^{1}H NMR spectrum of **3c** revealed the signals of the amide NH at 9.32 ppm, broad signal of aromatic protons of lidocaine moiety at 7.06 ppm, multiplet signals for CH, OH and CH_2 groups of gluconic acid between 4.04-3.26 ppm. Signals of the ethyl groups of lidocaine moiety were observed at 2.69 ppm (quartets of two CH_2 groups and the triplets of two CH_3 groups) were observed at 1.06 ppm. The ^{13}C spectrum of **3c** revealed two CO signals at 174.7 and 168.8 ppm. Four signals of the aromatic lidocaine moiety were found between 135.2-126.5 ppm. Nine ^{13}C signals of the CH, CH_2 and CH_3 groups were found between 73.0-11.8 ppm.

Organoleptic testing

The saccharinate and acesulfamate conjugates of lidocaine **3a** and **3b** were recrystallized from diethyl ether, dried under vacuum, and 5 mg doses were taken by one of the authors for organoleptic testing. Both acesulfamate **3a** and saccharinate **3b** effectively modulated the taste, since they were subjectively much less bitter than original lidocaine hydrochloride **1**. The sensation of **3a** and **3b** was sweet with a light bitter note similar to grapefruit, whereas lidocaine hydrochloride **1** evoked an unpleasant bitter sensation similar to Epsom salt.

Palatability assessment

Palatability was assessed using a modified protocol for the Orofacial Pain Assessment Device system, as described previously.[31] Briefly, rats were trained to drink sweetened condensed milk while making facial contact with a single thermode and lick-tube. During the training period (approximately 2 weeks) baseline licking events were recorded, and rats were considered ready for experimental testing once their baseline licking event average was greater than 1000 events. Rats were fasted for 13–15 h prior to testing. Compounds **3a** and **3b** and **1** as negative control were placed in three separate bottles and rats were randomly assigned to be tested on one of the three bottles. The number of reward licking events was recorded and used to compare the compounds. The results of this test demonstrated that lidocaine acesulfamate **3a** was the most palatable for rats, while the original lidocaine hydrochloride **1** was much less palatable, according to the number of licking events as shown in Fig. 2. Lidocaine saccharinate was inferior to both 3a and 1, which can be explained by a poor tolerability of saccharine by rats. It has been observed that rats do not like the taste of saccharine.[32]

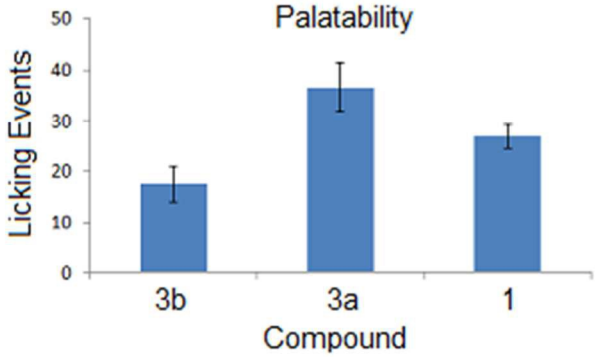


Fig. 2 Effects of taste modulated lidocaine formulations on palatability in rats

Local Anesthetic Properties

Hindpaw withdrawal latency

The effect of compounds **3a** and **3b** on local anesthesia in rats was evaluated using a reflexive measure of hindpaw withdrawal latency to a radiant heat source.[31] There was a significant treatment effect ($F_{4,53}$ = 51.28, P < 0.001) of the lidocaine derivatives on hindpaw withdrawal latency following thermal stimulation (**Figs.** 3-4). Two percent formulations of both **3a** and **3b** produced significantly higher hindpaw withdrawal latencies compared to baseline (naïve) and vehicle (water). When compared to the positive control lidocaine (2 %), there was no difference in response following **3a** or **3b** administration, indicating that both compounds were effective for producing a local anesthetic effect. We further characterized **3a** and found there was a significant dose effect ($F_{4,46}$ = 31.28, P<0.001), with doses ≥ 1% producing significantly higher latency times compared to vehicle and the lower concentrations (≤ 0.1 %).

Hindpaw withdrawal latency was significantly increased for **3b** (N=11), **3a** (N=10), and lidocaine hydrochloride **1** (N=10) compared to baseline (N=15) and vehicle (N=10) values.

The hindpaw withdrawal latency decreased significantly for a 2% water solution of **3c** and was approximately 12 sec.

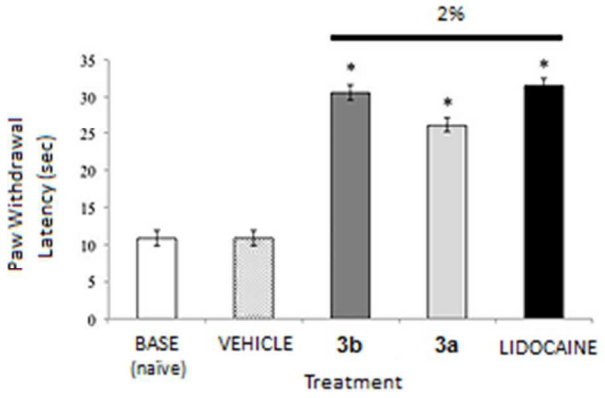


Fig 3 Effects of taste modulated lidocaine formulations on pain sensitivity.

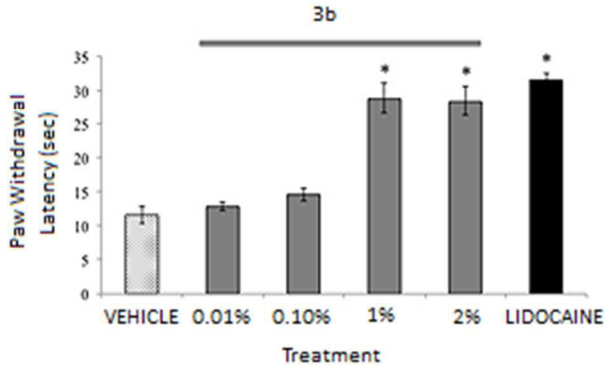


Fig. 4 Evaluation of **3b** indicated that doses ≥ 1% were needed to produce a significant local anesthetic effect.

Inhibition of Sodium Currents in Dissociated Trigeminal Ganglion Neurons

The mechanism of action of lidocaine requires the base to penetrate neuronal membranes to block the voltage gated sodium channels from the intracellular side of the cell membrane. To verify that the novel formulation did not disrupt membrane penetration, dissociated rat trigeminal ganglion neurons were whole cell voltage clamped and stimulated to produce inward sodium currents (Figure 5). Bath application of 57.5 μM of **3b** reduced sodium currents to 65.3% of control and 575 μM **3b** reduced sodium currents to 26.0% of control (N = 5 neurons). The currents returned to baseline levels within 10 min of washing out **3b** with fresh bath solution. These findings confirmed that the lidocaine moiety retained its action on voltage gated sodium channels in the new formulation using **3b**.

Safety of Taste Modulated Lidocaine Formulations

The objective was to determine the safety of lidocaine acesulfamate **3a** and lidocaine saccharinate **3b** compared to lidocaine hydrochloride **1** and physiologic saline (0.9% NaCl) 24 h after subcutaneous and gingival injections of 1.0 and 0.1 ml respectively of 2% solution (20 mg/ml) or saline. Three males and three female rats in each group received injections of saline (Group 1), lidocaine hydrochloride (Group 2), lidocaine saccharinate **3b, (**Group 3) or lidocaine acesulfamate **3a** (Group 4). At 24 h after injection the following treatment associated lesions were observed.

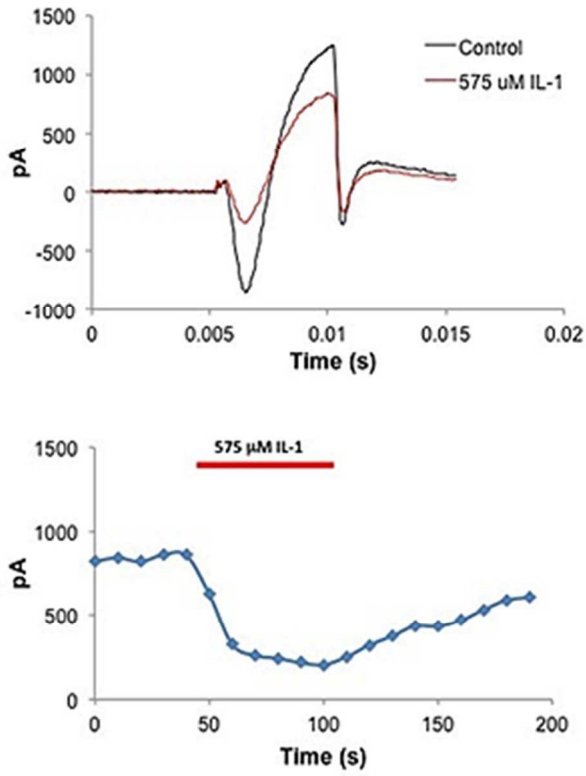


Fig. 5 Compound **3b** inhibits sodium currents in dissociated trigeminal ganglion neurons. Top: Representative voltage gated sodium currents in the presence and absence of **3b**. Bottom: time course of voltage gated sodium channel inhibition by **3b**. The red bar indicates the time that **3b** was applied *via* the bathing solution.

Injection of saline induced mild muscle degeneration and mild to moderate edema in and around the injection site. Two of the 6 rats showed mild inflammatory reactions and hemorrhage. In contrast, almost all rats receiving lidocaine analogs (Groups 2, 3, 4) developed inflammatory lesions characterized by infiltration by macrophages and lesser numbers of neutrophils in subcutis, submucosa and associated muscle. No differences in the severity or distribution of the lesions were noted among groups 2, 3 and 4. Lymphocyte apoptotic bodies in small to moderate numbers were noted in splenic periarteriolar lymphoid sheaths and lymphoid nodules in lymph nodes of a few rats in groups 3 and 4. No other treatment associated tissue lesions were found.

It was concluded that the taste modulated lidocaine formulations lidocaine acesulfamate **3a** and lidocaine saccharinate **3b** were no more toxic in inducing tissue damage at injection sites than currently FDA-approved lidocaine hydrochloride. Lymphocyte apoptosis that was infrequently seen could be secondary to glucocorticoid release associated with the stress of repeated anesthesia but warrants more careful study in future.

Conclusions

Substantial reduction of bitterness of lidocaine, the most widely used local anesthetic, was achieved by designing ionic conjugates of protonated lidocaine with anionic

sweeteners such as saccharinate and acesulfamate. This chemical approach differs greatly from the previous attempts to mask lidocaine hydrochloride's bitterness by physical mixing with sweeteners or other taste modifiers. By contrast in the proposed approach, chloride counter-ion is replaced by sweet taste anions. Such anion exchange provides water soluble formulations with zero osmolarity and higher bioavailability due to the use of more lipophilic counter-ions. Animal studies of lidocaine acesulfamate **3a** and lidocaine saccharinate **3b** showed that these compounds provided the same level of local anesthetic action and were as safe as lidocaine hydrochloride.

Acknowledgements
This work was supported by the Kenan Foundation (University of Florida).

Experimental
Materials and methods
^{1}H NMR spectra were recorded at 300 MHz and ^{13}C NMR spectra were recorded on Gemini spectrometer at room temperature. Chemical shifts are reported in ppm relative to TMS as internal standard (^{1}H NMR) or to the residual solvent peak (^{13}C NMR). The following abbreviations are used to describe spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, brs = broad singlet, dd = doublet of doublets. Elemental analysis was performed on a Carlo Erba-1106 instrument. High Resolution Mass Spectra were recorded using Thermo Scientific LCQ Ion Trap. Column chromatography was performed on silica gel (230–400 mesh). HPLC analysis was performed on Shimadzu liquid chromatographic system equipped with UV-detector. Detection was detected at 254 nm using Phenomenex Luna C18 reversed-phase column (250x4.6 mm id) 5 microns. All commercially available substrates were used as received without further purification.

*Synthesis of **3a** and **3b**:* To a solution of 2-((2,6-dimethylphenyl)amino)-*N,N*-diethyl-2-oxoethan-1-aminium chloride **1** (0.27 g, 1.0 mmol) in MeCN (15 mL) an equimolar quantity of sweetener sodium salt (sodium 6-methyl-1,2,3-oxathiazin-4-olate 2,2-dioxide 0.185 g for **3a** or sodium benzo[*d*]isothiazol-3-olate 1,1-dioxide 0.205 g for **3b**) was added and each mixture was stirred for 4h at the ultrasonic bath at 50 °C. After complete reaction (followed by TLC), each reaction mixture was filtered through 22 micron membrane filter and the filtrate was taken to dryness. Diethyl ether (3 x 25 mL) was added to the product and it was evaporated to give products **3a,b** in quantitative yields.

2-((2,6-Dimethylphenyl)amino)-N,N-diethyl-2-oxoethan-1-aminium 6-methyl-1,2,3-oxathiazin-4-olate 2,2-dioxide **3a**:
Colorless oil (98%, 0.389 g, 0.98 mmol). ^{1}H NMR (300 MHz, CD_3OD, δ): 7.12 (br s, 3H), 5.36 (s, 1H), 4.29 (s, 2H), 3.30 (q, *J* = 7.2 Hz, 4H), 2.24 (s, 6H), 2.01 (s, 3H), 1.34 (t, *J* = 7.2 Hz, 6H). ^{13}C NMR (75 MHz, CD_3OD, δ): 172.6, 165.0, 163.9, 136.8, 134.4, 129.3, 128.9, 102.4, 54.4, 50.8, 20.0, 18.7, 9.6. Anal. Calcd for $C_{18}H_{26}N_3O_5S$: C, 54.53; H, 6.61; N, 10.60. Found: C, 54.27; H, 6.83; N, 10.34. (+ESI-TOF) *m/z* for $C_{14}H_{23}N_2O$ $[M + 1]^+$ calcd. 235.1805, found 235.1815; (-ESI-TOF) *m/z* for $C4H4NO4S$ $[M - H]^-$ calcd. 161.9867, found 161.9869.

2-((2,6-Dimethylphenyl)amino)-N,N-diethyl-2-oxoethan-1-aminium benzo[d]isothiazol-3-olate 1,1-dioxide **3b**:
Colorless oil (99%, 0.413 g, 0.99 mmol). ^{1}H NMR (300 MHz, CD_3OD, δ): 7.73 (d, *J* = 6.3 Hz, 2H), 7.66 (q, *J* = 3.3 Hz, 2H), 7.09 (s, 3H), 4.86 (s, 3H), 4.26 (s, 2H), 3.32 (q, *J* = 7.5 Hz, 4H), 2.20 (s, 6H), 1.35 (t, *J* = 7.5 Hz, 6H). ^{13}C NMR (75 MHz, CD_3OD, δ): 170.2, 163.6, 143.8, 135.2, 133.4, 133.0, 132.3, 131.9, 127.9, 127.4, 123.2, 120.8, 53.0, 48.8, 18.3, 9.0. (+ESI-TOF) *m/z* for $C_{14}H_{23}N_2O$ $[M + 1]^+$ calcd. 235.1805, found 235.1815; (-ESI-TOF) *m/z* for $C_7H_4NO_3S$ $[M - H]^-$ calcd. 181.9917, found 181.9922.

*Synthesis of **3c**:*
An aliquot of 10 mL (12.20 g) of 50 % gluconic acid **2c** (31 mmol) was diluted to a 20 % solution and the solution was heated to 104 ± 4 °C. An equimolar amount of lidocaine free base **4** (31 mmol, 7.31 g) was dissolved in 25 mL of 95 % ethanol and added in 1 mL aliquots into the boiling gluconic acid. The pH of the solution changed from **2** (before addition of lidocaine) to 7, when 7 mL of lidocaine solution was added. After 5 h, when the pH dropped to 6, more lidocaine EtOH solution was added. Lidocaine aliquots were added within 24 h and after the mixture reached the pH of 7 it was taken to dryness in vacuum to give **3c**.

2-((2,6-Dimethylphenyl)amino)-N,N-diethyl-2-oxoethan-1-aminium (2R,3S,4R,5R)-2,3,4,5,6-pentahydroxyhexanoate **3c**:
Colorless oil (95%, 0.409 g, 0.95 mmol). ^{1}H NMR (300 MHz, DMSO-d_6, δ): 9.32 (s, 1H), 7.06 (s, 3H), 4.04 (d, *J* = 3.6 Hz, 1H), 3.92 (s, 1H), 3.60-3.56 (m, 3H), 3.48-2.91 (m, 6H), 2.69 (q, *J* = 7.2 Hz, 4H), 2.14 (s, 6H), 1.02-1.06 (m, 8H). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 174.7, 168.8, 135.2, 135.0, 127.7, 126.5, 72.9, 72.3, 71.6, 70.5, 63.4, 56.3, 48.2, 18.3, 11.8. Found (+ESI-TOF) *m/z* for $C_{14}H_{23}N_2O$ $[M + H]^+$ calcd. 235.1805, found 235.1808; (-ESI-TOF) *m/z* for $C_6H_{11}O_7$ $[M - H]^-$ calcd. 181.9917, found 181.9922.

Toxicity study method
Male and female Sprague Dawley rats, 76-100 g were received from Charles River Laboratories. Injection preparations were formulated as 2% solutions (20mg/ml), adjusted to 290-310 mOsm with NaCl and adjusted to pH 5.9 to 6.5 with NaOH before being filter sterilized with a 0.2 µm syringe filter. 0.9% NaCl (310 mOsm) and filter sterilized was used as a vehicle control.

Rats (3 male and 3 female rats for each solution) were anesthetized with isoflurane and were injected using a 0.5 insulin syringe with 29 gauge needle in the mucobuccal fold above the root apex of the first right upper molar with 0.1 ml of solution. The cervical subcutaneous tissues were injected with 1.0 ml of solution using a syringe with 22 gauge needle.

Rats were necropsied at 24 hours after injection following isoflurane anesthetic overdose and exsanguination, and the following tissues were fixed and examined histologically: Skull at injection site, cervical skin and subcutaneous tissues at injection site, bone marrow, eye, liver, spleen, kidney, adrenal gland, larynx, esophagus, salivary gland,

submandibular lymph node, small intestine, large intestine, stomach, pancreas, mesenteric lymph node, gonad (ovary or testicle), bladder, heart (left and right ventricle), lung and brain (cerebrum, thalamus, cerebellum, medulla).

Preparation of Solutions Injected

10ml of each solution needed for Safety study

1. Lidocaine HCl for each ml of injectable drug

20mg – Lidocaine HCl

1 ml 0.60% NaCl aqueous

10 microliters 1 N NaOH

Filter with 0.2 micrometer filter

After Filtering: 6.3 pH (pH paper measurement); 290 mOsm (Measured by vapor pressure method)

Attempts to adjust pH further resulted in drug precipitation near pH 7.4.

2. **3a** (Lidocaine acesulfamate) for each ml of injectable drug

20mg - Lidocaine acesulfamate

1 ml 0.65% NaCl aqueous

6 microliters 1 N NaOH

Filter with 0.2 micrometer filter

After Filtering: 5.9 pH; 320 mOsm

4. Saline - 0.9% NaCl aqueous

Filter with 0.2 micrometer filter

After Filtering: 5.5 pH; 310 mOsm

3. **3b** (Lidocaine saccharinate) for each ml of injectable drug

20mg - Lidocaine saccharinate

1 ml 0.65% NaCl aqueous

8 microliters 1 N NaOH

Filter with 0.2 micrometer filter

After Filtering: 6.5 pH; 298 mOsm

Thermal pain testing. Male Sprague Dawley rats (200-300g, Harlan Labs) were housed in groups of two and were maintained in a standard 12-h light/dark cycle and testing was completed in the light portion of the cycle between 09:00-12:00. Animals were placed into the behavioral procedure room 30 min prior to testing to acclimate. When not in testing sessions, food and water were made available ad libitum. Animal testing procedures complied with the ethical guidelines and standards established by the University of Florida's Institutional Animal Care & Use Committee and with the Guide for Care and Use of Laboratory Animals (National Research Council Guide for the Care and Use of Laboratory Animals. Washington, D.C., National Academy Press; 1996.)

Response to hindpaw heat pain was determined by placing unrestrained animals on a clear glass platform under a small plastic cage and animals were habituated for 5 min. A radiant heat source was aimed directly under the ventral hindpaw surface and the time to paw withdrawal was recorded as described previously.[32] Baseline responses were obtained under naïve conditions (*e.g.*, no injection), while post-treatment effects of the lidocaine, lidocaine derivatives (**3a**, **3b**), and vehicle (water) was assessed 10 minutes following plantar injection (100 µl). A cutoff of 32 sec was used to prevent tissue damage. Statistical analyses: an analysis of variance was used to evaluate the effects of treatment on hindpaw withdrawal latency (SPSS Inc). When significant differences were found, post-hoc comparisons were made using the Tukey post-hoc test. *$P < 0.05$ was considered significant in all instances.

Electrophysiology. Rats (N=3) were anesthetized with 5% isoflurane in O_2 and decapitated. The trigeminal ganglia were carefully removed and placed in Tyrodes buffer containing 1mg/ml collagenase (Sigma). Tyrodes buffer consisted of (mM) 140 NaCl, 4KCl, $2MgCl_2$, $2CaCl_2$, 10 glucose, and 10 HEPES, adjusted to pH 7.4 with NaOH. The ganglia were incubated at 37 ^oC for 2 hours and then dissociated by trituration with a plastic pipette. The cells were then pelleted by centrifugation at 100g for five minutes and resuspended in fresh Tyrodes. The suspended cells were plated onto 30 mm poly-D-lysine coated culture plates and allowed to adhere to the plates for one hour prior to experiments. The plates were placed on an inverted microscope and continuously superfused with fresh room temperature Tyrodes buffer. Cells were whole cell voltage clamped using glass pipettes pulled to 2-4 MΩ and filled with electrode buffer consisting of (mM) 140 KCl, $1CaCl_2$, $2MgCl_2$, 10EGTA, 10HEPES, adjusted to pH=7.4 with KOH. The amplifier (Axopatch 200B, Axon Instruments) was adjusted to compensate for cell capacitance and resistance was 60-70% compensated.[33] Cells were held at -60 mV and voltage gated sodium currents were evoked every 10 seconds by stepping the voltage to +20 mV for 5 ms. All data was digitized and collected on a computer for analysis. **3b** was bath applied to the cells while recording the currents.

Palatability assessment. Male Sprague–Dawley rats (n=30, 12-16 weeks old, Charles River, Raleigh, NC) were maintained in a standard 12-h light/dark cycle and were allowed access to food and water ad libitum when not being tested. Rats' weights were recorded every week to monitor general health. Animal testing procedures and general handling complied with the ethical guidelines and standards established by the Institutional Animal Care & Use Committee at the University of Florida (Institute of Laboratory Animal Resources, 1996).

Notes and references

[a] Department of Chemistry and Physics, Georgia Regents University, Augusta, GA 30904

[b] Center for Heterocyclic Compounds, Department of Chemistry, University of Florida, Gainesville, FL 32611

[c] EigenChem Technologies Inc., Alachua, FL 32615

[d] Department of Medicine, College of Medicine, University of Florida & Shands, Gainesville, FL 32610

[e] Department of Orthodontics, College of Dentistry, University of Florida, Gainesville, FL 32610

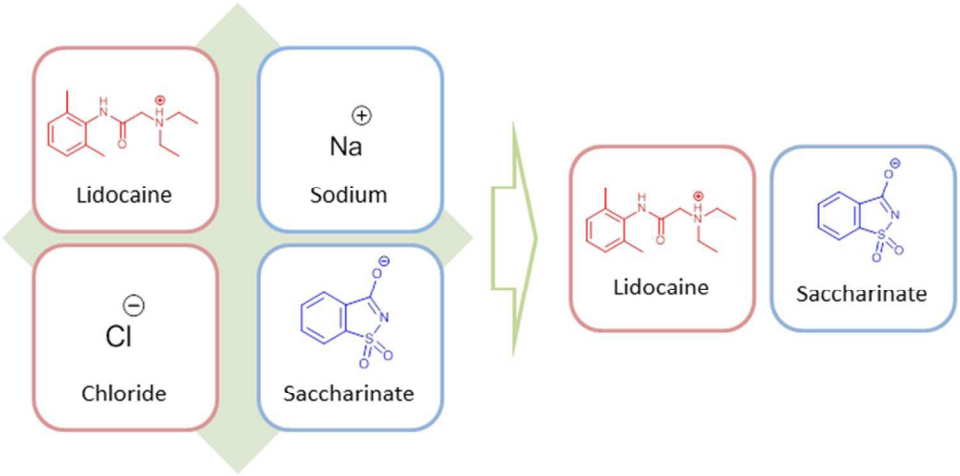
[f] Department of Oral and Maxillofacial Surgery, College of Dentistry, University of Florida, Gainesville, FL 32610

[g] Department of Infectious Diseases and Pathology, College of Veterinary Medicine University of Florida, Gainesville, FL 32611

[h] Altschuler Periodontic and Implant Center, Gainesville, FL 32606
[i] Department of Pathology, Immunology and Laboratory Medicine, College of Medicine, University of Florida, Gainesville, FL 32610

† Electronic Supplementary Information (ESI) available: ^{1}H, ^{13}C and HRMS data for all the synthesized products **3a–3c**. See DOI: 10.1039/b000000x/

1 S. F. Malamed, in *Handbook of Local Anesthesia*, Mosby, St. Louis, Missouri, 6-th edn., 2012, p 432.
2 S. Kohl, M. Behrens, A. Dunkel, T. Hofmann and W. Meyerhof, *J. Agric. Food Chem*., 2013, **61**, 53.
3 V. Vummaneni and D. Nagpal, *Int. J. Res. Pharm. Biomed. Sci*., 2012, **3**, 510.
4 *US Pat*., 8 362 083.
5 *US Pat*. 4 975 465.
6 *US Pat*. 6 540 978.
7 P. Leksrisompong, P. Gerard, K. Lopetcharat and M. Drake, *J. Food Sci.*, 2012, **00**, S282.
8 K. Tomoda, M. Asahiyama, E. Ohtsuki, T. Nakajima, H. Terada, M. Kanebako, T. Inagi and K. Makino, *Colloids Surf. B.*, 2009, **71**, 27.
9 A. M. Suthar and M. M. Patel, *J. Pharm. Sci. Tech*., 2010, **2**, 303.
10 F. M. Mady, A. E. Abou-Taleb, K. A. Khaled, K. Yamasaki, D. Iohara, T. Ishiguro, F. Hirayama and K. M. Uekama, *J. Pharm. Sci*., 2010, **99**, 4285.
11 Q. Tan, L. Zhang, L. Zhang, Y. Teng and J. Zhang, *Chem. Pharm. Bull*., 2012, **60**, 1514.
12 M. K. Jeffcoat, N. C. Geurs, I. Magnusson, S. R. MacNeill, N. Mickels, F. Roberts, P. Robinson, A. Salamati and Yukna, R., *J. Periodontol*., 2001, **72**, 895.
13 E. H. Y.Batawi, *Eur. Arch. Pediatr. Dent*., 2013, **14**, 381.
14 S. Mogensen, C. Treldal, E. Feldager, S. Pulis, J. Jacobsen, O. Andersen and M. Rasmussen, *Local Reg. Anesth*., 2012, **5**, 17.
15 M. A. Bogle, A. K. Joseph and D. MacFarlane, *Surgical Gem*., 2004, **30**, 792.
16 J. L. Benumof, *Anesthesiology*, 1997, **87**, 1598.
17 *US Pat*. 6 949 264.
18 W. L. Hough and R. D. Rogers, *Bull. Chem. Soc. Jpn*., 2007, **12**, 2262.
19 W. L. Hough, M. Smiglak, H. Rodríguez, R. P. Swatloski, S. K. Spear, D. T. Daly, J. Pernak, J. E. Grisel, R. D. Carliss, M. D. Soutullo, J. H. Davis and R. D. Rogers, *New J. Chem*., 2007, **31**, 1429.
20 J. Stoimenovski, D. R. MacFarlane, K. Bica and R. D. Rogers, *Pharm. Res*., 2009, **27**, 521.
21 K. Bica, H. Rodríguez, G. Gurau, O. A. Cojocaru, A. Riisager, R. Fehrmann and R. D. Rogers, *Chem. Commun*., 2012, **48**, 5422.
22 G. E. DuBois and I. Prakash, *Annu. Rev. Food Sci. Technol*., 2012, **3**, 353.
23 C. B. Romanuk, Y. G. Linck, A. K. Chattah, G. A. S. L. Monti, Cuffini, M. T. Garland, R. Baggio, R. H. Manzo and M. E. Olivera, *Int. J. Pharm.*, 2010, **391**, 197.
24 *US Pat*. 6 576 260.
25 P. Nockemann, B. Thijs, K. Driesen, C. R. Janssen, K. Van Hecke, L. Van Meervelt, S. Kossmann, B. Kirchner and K. Binnemans, *J. Phys. Chem. B*, 2007, **111**, 5254.
26 M. Pietruska, A. Paniczko and D. Waszkiel, *Adv. Med. Sci*., 2006, **51**, 162.
27 W. S. Lyra, F. A. C. Sanches, F. A. S. Cunha, P. H. G. Diniz, S. G. Lemos, E. C. Silva and M. C. U. Araujo, *Anal. Methods*, 2011, **3**, 1975.
28 B. Michael, S. Fishbane, D. W. Coyne, R. Agarwal and D. G. Warnock, *Nat. Clin. Prac. Nephr*., 2006, **2**, 92.
29 C. P. Thakur and S. Narayan, *Annals Trop. Med. Parasit*., 2004, **98**, 129.
30 *US Pat*. 2 049 442.
31 Anderson E. M, Mills R, Nolan T. A, Jenkins A. C, Mustafa G, Lloyd C, Caudle R. M, Neubert J. K. J Vis Exp. 2013, **76**,:e50336.
32 Dr. John Neubert, private communication.
33 Neubert, J. K.; Rossi, H. L.; Pogar, J.; Jenkins, A. C.; Caudle, R. M. *Behav. Brain Func*. 2007, 3:49.
34 K. Hargreaves, R. Dubner, F. Brown, C. Flores and J. Joris *Pain*, 1988, **32**, 77.
35 R. M. Caudle, L. Karai, N. Mena, B. Y. Cooper, A. J. Mannes, F. M. Perez, M. J. Iadarola and Z. Olah, *Neurotoxicology*, 2003, **24**, 895.



Ionic conjugation of lidocaine with artificial sweeteners creates a new type of taste modulated materials for dentistry