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Development of Dirhodium Sugar-Substituted Carboxylate and Acetamide Complexes and Evaluation of their Potential as Lectin Inhibitors

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Development of Dirhodium Sugar-Substituted Carboxylate and Acetamide Complexes and Evaluation of their Potential as Lectin Inhibitors

BY BETHANY MASTEN

Bethany is a senior majoring in Chemistry. She was an ATP Summer Grant recipient. She was aided in her research by her mentor, Dr. Stephen Haefner. She plans to attend graduate school to study green chemistry and will try to save the world, provided she isn't in the poor house.

Abstract:

A current interest at the interface of biology and chemistry is the development of molecular and polymeric species that can function as scaffolding for pendant sugar groups. Such materials have the ability to affect biological phenomena including lectin-based cellular binding. Compounds that can inhibit this binding have the potential to prevent tumor metastasis. This project involved the synthesis of dirhodium carboxylate compounds with pendant saccharide moieties, as well the development of a synthetic method to prepare sugar derivatives of dirhodium acetamides; compounds that are potential lectin inhibitors. Several methods for preparing glucuronate and N-acetyl glucosamine complexes of dirhodium (II) were examined. One pathway to the synthesis of these compounds involves reacting a Rh (III) precursor to form a Rh-Rh bond while substituted carboxylates act as supporting bridges. This pathway was investigated and prior results were found to be irreproducible. Reaction of sodium glucuronate or glucuronic acid with $RhCl_3 \cdot xH_2O$ failed to produce any dirhodium glucuronate species possessing a Rh-Rh bond. Reactions of dirhodium tetraacetate with glucuronic acid, however, produced partially substituted species in which the dirhodium bond is maintained as evidenced by UV-visible spectroscopy. Electronic tuning of the leaving group was attempted through the use of dirhodium tetratriflouroacetate which, when reacted with sodium glucuronate, yielded partial substitution of the trifluoroacetate groups as seen with infrared-, ^{1}H-, and ^{19}F- NMR- spectroscopies. A preliminary lectin-binding assay for this partially-substituted product showed no inhibitory effects. Substituted dirhodium acetamide synthesis was also attempted from N-acetyl glucosamine

and dirhodium tetraacetate. Products characterization through infrared and ^{1}H NMR spectroscopies once again failed to show conversion.

One of the more important recent developments regarding sugars occurred in the 1960's. During this time it was determined that saccharides are not confined to simple ring and chain structures, but are instead capable of complex interactions with other biomolecules and sugars.[1,2] The interactions most relevant to life are those of the sugars found on the lipids and proteins on the surfaces of cells. These complexes, respectively named glycolipids and glycoproteins (for which oligosaccharides are the sugar components), play essential roles in cell-cell adhesion, recognition, inflammation, and immune defense.[1,2] Further, it was found that sugars on the cellular surfaces are commonly arranged in groups, or clusters.[3,4] These clusters are presented in specific arrangements, and as such are capable of binding with other sugars found in complementary arrangements. Biomimetically, artificial scaffolds are also used to present pendant sugar moieties in a desired arrangement. Multiple stabilizing structures have been designed and studied including rigid organics,[5] dendrimers,[6] glycoconjugates,[7] and metal ions.[7-10] Other studies have investigated the effects of pendant sugars, finding significant bioactivity in the form of anti-tumor, antifungal, and antibacterial actions.[11,12] The glycoconjugate study, in addition, found that varying either the scaffold or the sugar substituents causes a change in the bioactivity.[7] A proposed mechanism for these variations involves an alteration to the spaces between the substituent groups, sometimes referred to as "pockets". Knowing that the bioactivity of each complex can be controlled through changes to the scaffold and/or the pendant saccharide, the topological arrangements of these carbohydrate groups becomes very significant from a biological standpoint. This is because the saccharide substituents are arranged in a similar "clustered" manner to the oligosaccharides that interact with lectins (sugar-binding proteins). With the pendant sugars acting in the same fashion as the oligosaccharides, competitive binding may occur as both groups try to coordinate with the lectins. A dirhodium acetamide scaffold could present multiple arrangements of sugar substituents, and may therefore provide multiple possibilities for lectin interactions and inhibition of lectin binding.

Lectins, as previously mentioned, are proteins that bind to the end of oligosaccharides on cellular exteriors. Lectins commonly bind to more than one sugar unit at a time and as such, they are a method by which cells agglutinate, or clump together.[13] The presence of multiple clusters of oligosaccharides on the surface of the cell increases the lectins' ability to bind in a multivalent manner. Any substance, therefore, that has similar carbohydrate-coordinating abilities (i.e. the ability to arrange its pendant sugars as clustered units) will be able to interact in a similar manner with the lectins, perhaps coordinating with the lectins in place of the oligosaccharides. The potential applications for this ability are numerous. Viruses and metastatic cancer cells, for example, are required to bind to the oligosaccharides on healthy cells before infecting them, so an agent that already occupies these sites has the potential to prevent infection.

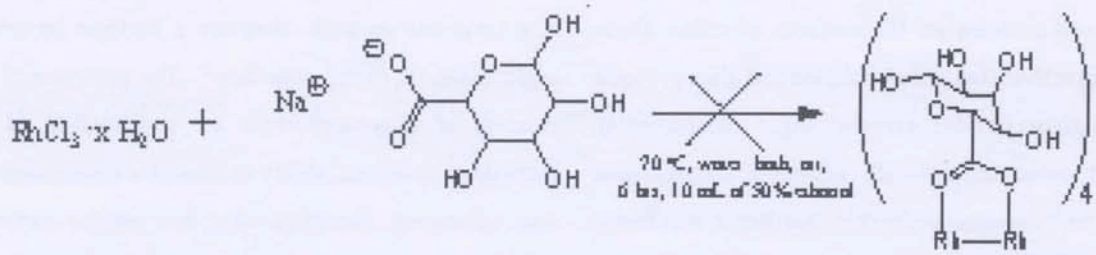
Furthermore, it has been found that lectins play a role in inflammation and cell-cell signaling;[1,2] in instances where this is disadvantageous to an organism, lectin-binding inhibitors would be of great potential use. The dirhodium compounds that this study investigated were likely to have the ability to act as lectin binding inhibitors, and thus had potential for the aforementioned applications. Anti-tumor activity is another characteristic of dirhodium complexes, and such activity has been suggested to increase with complexation to sugar-modified carboxylates.[11, 12] This finding evokes the idea that other sugar modified dirhodium complexes, such as those investigated here, may also show anti-tumor properties, though this study only looked at lectin inhibition as an indicator of such potential.

Results and Discussion

Dirhodium Carboxylate Complexes

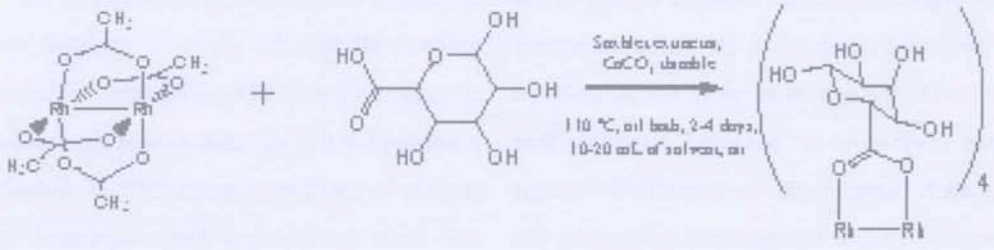
Eq 1

Much of the initial synthetic work focused on attempting to reproduce the work of De Souza Gil, who reported a simple process for attaching sugar-substituted carboxylic acids to a rhodium(II) fragment, with promising results (Equation 1).[11] In this reaction, the Rh(III) precursor is reduced to Rh(II) with concomitant formation of the Rh-Rh single bond. The carboxylates act as bridges, assisting with the formation of the metal-metal bond.



Rhodium trichloride was heated with the sodium salt of glucuronic acid for 6 hours, and a slight color change was observed, from brown to greenish-brown. To separate the mixture, the reaction was run through a Sephadex G25-50 column as reported by De Souza Gil.[11] Complete separation was, however, not observed. The solution did partially separate into a band that was more yellow/brown and another that had a green/brown color to it. However, the characteristic teal color associated with dirhodium tetracarboxylates was missing, suggesting that the synthesis was unsuccessful. A similar reaction with the $RhCl_3 \cdot xH_2O$ complex was also attempted using glucuronic acid instead of sodium glucuronate. This resulted in no observed color change after the initial formation of the orange/brown solution, despite 24 hours of heating. Consequently, the rhodium trichloride pathway was abandoned in favor of a route in which the starting material already contained the Rh-Rh bond.

Eq 2

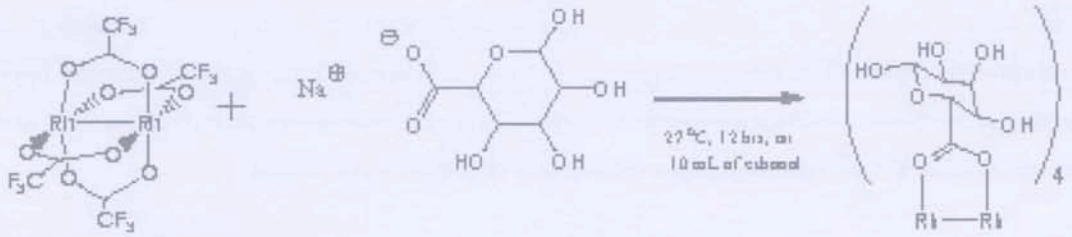


Earlier work has shown that many dirhodium tetracarboxylates can be prepared from dirhodium tetraacetate through ligand exchange.[14] Dirhodium acetate was refluxed with 10 molar equivalents of glucuronic acid in a Soxhlet extractor (Equation 2).

The thimble of the extractor was loaded with $CaCO_3$ to neutralize the volatile acetic acid that would be produced in the reaction. A variety of solvents were explored with ethanol found to be the most promising. Refluxing the solution for 6 days resulted in a blue-teal solution. The solvent was removed under vacuum and the resulting solid was redissolved in H_2O. Elution of the aqueous solution down a Sephadex column produced two bands, one yellow-brown and the other teal in color. The teal band was allowed to slowly evaporate, yielding a highly hygroscopic teal solid. The IR spectrum showed a major absorbance at 1600 cm^{-1} shifted from the 1580 cm^{-1} signal found in dirhodium acetate's spectrum, and indicating some conversion. Another absorbance at 1710 cm^{-1} is shifted only slightly from the 1705 cm^{-1} signal of glucuronic acid. Hydroxyl groups are also present, as evidenced by a strong, broad absorbance at 3403 cm^{-1}. The ^1H NMR spectrum suggested that bound acetate was still present along with ligand. The acetate was shown by a resonance at 2.11 ppm. Further suggested by NMR spectra is the presence of a mixture of compounds, as shown by multiple sets of resonances, especially as sets of multiplets between 3.95 and 3.09 ppm. Based upon the spectroscopic data, it is clear that the substitution of glucuronate for acetate was incomplete.

The lack of complete substitution of the bridging acetate groups prompted us to use an electronically more favorable leaving group, namely trifluoroacetate ($CF_3CO_2^-$). The highly electronegative fluorine substituents withdraw electron density from the oxygen atoms and substantially weaken the Rh-O bond. Reaction of dirhodium tetratrifluoroacetate with four equivalents of sodium glucuronate in water at room temperature for 12 hours produced a green solution (Equation 3).

Eq 3

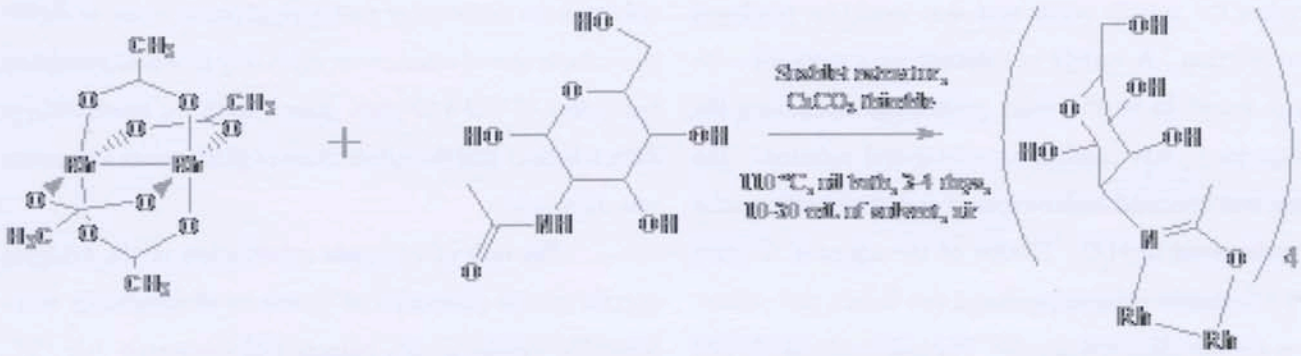


Removal of the water followed by repeated washings with CH_2Cl_2, ether, and acetonitrile resulted in a green solid. The IR- and NMR spectra of the resulting product were more promising than previous samples, indicating low levels of impurities and the presence of bound sugar (all free N-acetyl glucosamine should have been removed by the acetonitrile washings). The IR spectrum confirms the presence of sugars with a broad O–H absorption near 3400 cm^{-1}. The set of multiplets in the ^1H NMR between 3.10-3.68 ppm is evidence for bound glucuronate as well. The clarity of the multiplet resonances between 3.10-3.68 ppm suggests the presence of only one compound. Complete ligand exchange did not occur, however, since the presence of bound trifluoroacetate was confirmed by a ^{19}F NMR resonance at -75.5 ppm. The relative purity of this sample, as well as the evidence for at least partial substitution, led us to select it for subsequent lectin testing (*vide infra*).

Dirhodium Acetamide Complexes

As with the prior glucuronic acid reactions, a Soxhlet extractor was used for the dirhodium acetamide syntheses (Equation 4). Calcium carbonate was added to the thimble to neutralize and remove from solution any evolved acetic acid. A number of solvents for the reaction were examined. Toluene and ethanol were our first choices as solvents in order to avoid dissolving the calcium acetate that would be produced as a result of using the thimble.

Eq 4



Although the solutions eventually acquired a teal color, toluene did not initially dissolve either the dirhodium acetate or the N-acetyl glucosamine. Deionized water was by far the least successful solvent: it initially dissolved everything, leading to incomplete reactions. The initial teal solutions turned dark brown within one day of refluxing indicating decomposition of the Rh-Rh bond. When ethanol was used, the resulting solid could be dissolved in water and run down a Sephadex G25-40 column to separate teal bands from yellow/brown bands that may have resulted from some sugar decomposition. The products of the toluene reactions were not water soluble enough to allow for column chromatography, but did result in purple solutions when washed with acetonitrile. The purple washings were dried and remaining solids determined to be dirhodium acetate. Subsequent IR and ^{1}H NMR spectra of these solids revealed that much of the extra ligand was still present, and that the initial rhodium acetate was present as well. There may have been a partial replacement of one or two of the acetates by acetamides, but the spectra shows a definite lack of complete conversion.

Lectin Inhibition Study

Despite our failure to isolate a completely substituted dirhodium glucuronate or acidamidate, we still wanted to test for lectin inhibition to investigate the properties of the partially substituted species. To this end, a simple agglutination assay was used to determine the lectin inhibiting potential of the complex formed by the reaction of dirhodium tetratrifluoroacetate with sodium glucuronate. This assay verified that the presence of the dirhodium compound did not cause a decrease in the number of red blood cells that agglutinate compared with a positive and negative control. The results of this study suggest that the dirhodium complex does not inhibit lectin binding. This was shown by a hemagglutination assay with human erythrocytes in which concanavalin A (Con A) was used as the lectin (Figure 1).

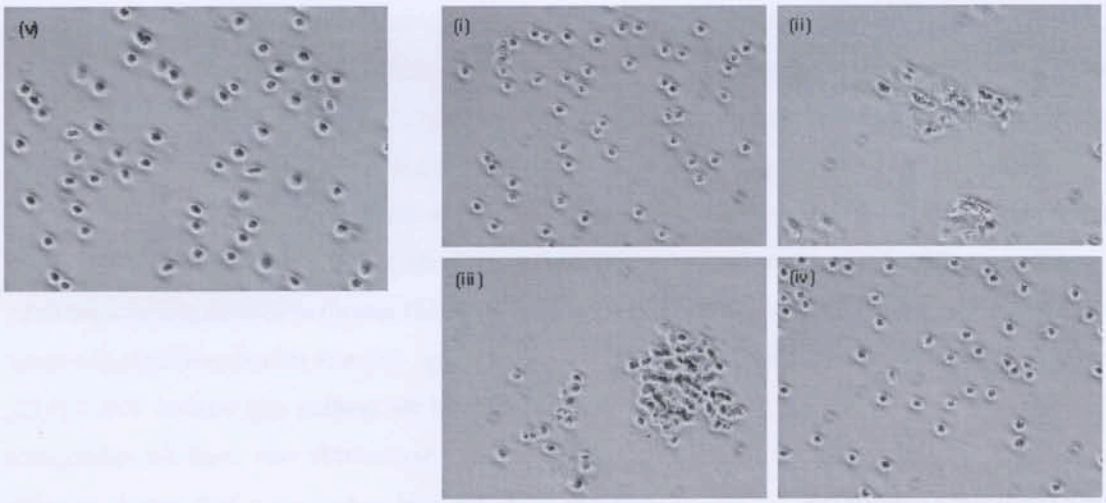


Figure 1: Light microscopy images from an Olympus BX51 microscope taken in phase mode with a 40x objective lens: (i) Human erythrocyte suspension in buffer with no lectin present. (ii) Erythrocytes in buffer with concanavalin A (Con A) lectin showing 68% agglutination. (iii) Erythrocytes, Con A, and dirhodium complex, showing 53% agglutination. (iv) An example of lectin inhibition, caused by the presence of mannose. (v) A control containing only erythrocytes and dirhodium complex, showing a lack of agglutination.

In contrast to the 2% of cells agglutinating with both the control (only cells) and a sugar (mannose) inhibited sample, the rhodium carboxylate allowed 53% of the red blood cells to agglutinate. This clearly shows that our product did not inhibit the lectin-induced binding. This is most likely the result of incomplete complexation with the glucose-modified acetates.

Conclusion

Of the investigated methods for preparing glucuronate complexes of dirhodium, there was no success when a Rh(III) precursor was used. That pathway was therefore abandoned in favor of using a starting material that already contained the rhodium-rhodium bond: dirhodium tetraacetate. Incomplete conversion, as evidenced by UV-visible-, IR-, and NMR spectroscopies, led us to use more reactive species: sodium glucuronate and dirhodium tetratrifluoroacetate. This led to partial substitution of the products, which unfortunately were found to exhibit no lectin-inhibiting properties. Although partial substitution was accomplished with the dirhodium carboxylate compounds, there was no such success with the dirhodium acetamides. Different synthetic processes will be attempted to complex the dirhodium acetamide with sugars, including reacting $Rh_2(OAc)_4$ with the molten ligands. The presence of the hydroxyl groups leads to the undesirable decomposition of the sugars upon melting. This can be circumvented by acetylating the hydroxyl groups on the N-acetyl glucosamine, which can then be used directly in a molten reaction. Following the metal complexation, these acetyl group can then be removed by treatment with NaOMe.[5] The protecting acetyl groups help to stabilize the compounds both thermally and chemically so that they will not decompose upon melting. Alternatively, the protected acetamides can be deprotonated and reacted with $RhCl_3$ or $Rh_2(OAc)_4$.

Experimental Section

General Considerations

All chemicals and solvents were purchased from commercial sources and used without further purification,

including acetonitrile, diethyl ether, ethanol, isopropyl alcohol, and toluene. All visible spectra were gathered using a Hewlett-Packard 8543 diode-array spectrophotometer. Infrared spectroscopy was performed as between sodium chloride plates in a Nujol mull. ^{1}H- NMR studies were performed on either a 400 or a 500 MHz JEOL-ECX spectrometer.^{19}F NMR spectrum was performed on a 400 MHz JEOL-ECX spectrometer with shifts reported relative to CCl_3F.

Synthesis of dirhodium carboxylate complexes

Method 1: The literature method reported by De Souza Gil[11] required mixing 0.100 g (0.44 mmol) of $RhCl_3$·x H_2O with 0.216 g (0.92 mmol) of the sodium glucuronate or 0.179 g (0.92 mmol) of glucuronic acid. The reactions were performed under argon on a vacuum line using Schlenk techniques. 10 mL of 50% ethanol was added resulting in an orange-brown solution in both reactions. The sodium glucuronate reaction was stirred in a 70° C water bath for 6 hours. The glucuronic acid reaction was stirred in a 70° C water bath for 24 hours. A G25-40 Sephadex column was used in to purify the sodium glucuronate solution, and was eluded with a 50% methanol solution. A yellow solution came out of the column, and a darker green band was immobilized on the column and was later pulled through with deionized water. The resulting olive green solution was put through the column again, but did not separate into discrete bands.

Method 2: In a Soxhlet extractor whose thimble was filled with $CaCO_3$, 0.100 g (0.20 mmol) $Rh_2(OAc)_4$· 2 CH_3OH was mixed with 0.384 g (2.00 mmol) of glucuronic acid. 15 mL of ethanol were added and the solution was stirred in an 110° C oil bath for 6 days. Solution color remained the initial teal/aqua throughout heating with some white solid remaining undissolved. The solvent was removed under vacuum and the resulting solid redissolved in deionized water. This teal solution was run through a G25-40 Sephadex column eluded with deionized water, resulting in yellow and teal bands. The teal band was allowed to slowly evaporate over 7 days, yielding a sticky teal solid. UV visible spectrum (water) λ_{max}, nm: 449, 587. IR (cm^{-1}): 3445, 2924, 2854, 1755, 1456, 1377, 1038. ^{1}H NMR (400 MHz, D_2O, δ): 1.79 (s), 2.11 (s), 3.96-3.09 (m), 4.25 (s), 4.46 (d), 5.04 (d), 5.35 (s).

Method 3: Dirhodium trifluoroacetate (0.100 g) was mixed with 0.143 g (0.61 mmol) of sodium glucuronate in 15 mL of deionized water. This was stirred overnight, the water was pumped off, and the residue was washed with CH_2Cl_2 and diethyl ether. Acetonitrile was used for subsequent washings, with the solid and solution both purple in color. IR (cm^{-1}): 2924, 2854, 1463, 1377, 1084. ^{1}H NMR (400 MHz, D_2O, δ): 1.79 (s), 3.10-3.68 (m), 3.97 (d, J=10 Hz), 4.4627 (d, J=7.8 Hz), 4.52 (d, J=7.8 Hz), 5.04 (d, J=3.7 Hz), 5.12 (d, J=3.6 Hz). ^{19}F NMR (400 MHz, D_2O, δ): -75.5 (s).

Attempted synthesis of dirhodium acetamide complexes

In a Soxhlet extractor equipped with a cellulose thimble filled with $CaCO_3$, 0.100 g $Rh_2(OAc)_4$· 2 CH_3OH and 0.4371 g N-acetyl glucosamine were mixed. 10 mL of toluene was added as a solvent, and the reaction was refluxed via heating mantle for 4 days. The resulting solution was pumped down and water was added, into which the solid did not readily dissolve. The water decantings were then pumped down and acetonitrile was used to wash the solid resulting in a purple solution. This solution was decanted and the solvent was slowly evaporated to give a purple solid that became green in the presence of moisture. Spectral properties of the solid were consistent with those of dirhodium acetate.

Agglutination Assay

The concanavalin-A (Con-A) lectin was from jack bean and used as received from a commercial source (Modern Biology). The assay was performed in a buffer solution made up of 0.15 M NaCl, 0.1 mM $MnSO_4$, 0.1 mM $CaCl_2$, 0.2% BSA and 10 mM tris-HCl at a pH of 6.8. Two drops of

human blood were obtained and mixed with 2 mL of buffer resulting in an erythrocyte suspension. Five samples were prepared according to the following chart:

Tube #	Erythrocyte Suspension (μL)	Buffer (μL)	Con A Lectin (μL)	Rhodium carboxylate complex (μL)	Mannose (μL)
1	15	20	0	0	0
2	15	10	10	0	0
3	15	0	10	10	0
4	15	0	10	0	10
5	15	10	0	10	0

The slides were incubated at room temperature for 45 minutes with gentle mixing approximately every 10 minutes. At the conclusion, a 10 μL sample from each tube was analyzed using light microscopy images from an Olympus BX51 microscope taken in phase mode with a 40X objective lens by counting 100 erythrocytes and the number agglutinated (touching each other) for each sample. Results were: tube 1, 2%; tube 2, 68%; tube 3, 53%; tube 4, 13%; tube 5, 2%.

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