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An efficient method for the preparation of peracetylated Neu5Ac2en by flash vacuum pyrolysis

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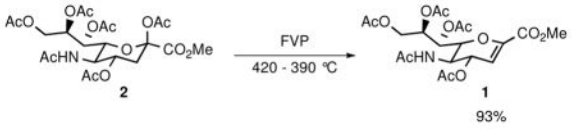
Abstract



Peracetylated Neu5Ac2en methyl ester, an intermediate in the synthesis of the influenza neuraminidase inhibitor Relenza, has been synthesized in high yields from peracetylated Neu5Ac methyl ester by flash vacuum pyrolysis. Mechanistic evidence including deuterium labeled studies and DFT (B3LYP) calculations suggest this transformation proceeds via an intramolecular synelimination.

The sialic acid Neu5Ac, found naturally as a cell surface carbohydrate on mammalian cells, is involved in a number of vital biological processes such as cellular recognition and adhesion, and viral receptor recognition.[i,ii] For these reasons, derivatives of Neu5Ac have been of general interest to researchers, as biological probes and cell surface enzyme inhibitors. The commercial influenza neuraminidase inhibitor Relenza (4-deoxy-4-guanidino-Neu5Ac2en) is one such derivative. Developed by von Itzstein and co-workers and put on the market jointly by Biota Ltd. and Glaxo-Smith-Klein this drug is one of 2 neuraminidase inhibitors presently on the market for treatment of the symptoms associated with influenza. Currently Relenza is being stockpiled around the world in preparation for any potential outbreak of avian influenza.

The 2–3 unsaturation present in Relenza is a common structural motif found in a number of sialic acid derivatives possessing neuraminidase binding activity. It is also a useful synthetic handle for the preparation of sialic acid derivatives functionalized at C-3.[iii] For these reasons several methods have been developed for the preparation of the glycal, Neu5Ac2en (**1**). These include the most commonly used β-elimination of peracetylated Neu5Ac glycosyl chloride, catalyzed by Et_3N[iv] or DBU,[v] and TMSOTf catalyzed elimination of the peracetylated methyl ester of Neu5Ac (**2**). More recent advances in the preparation of **1**

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Supporting Information Available: Experimental procedures including a detailed description of our FVP apparatus, computational data, and representative ^{1}H NMR spectra of crude FVP product are available free of charge via the Internet at http://pubs.acs.org

include oxidation[vi] or elimination[vii] of peracetylated Neu5Ac thioglycosides, and β-elimination of the peracetylated glycosyl chloride methyl ester in refluxing Na_2PO_4. However, these recent syntheses rely on activating Neu5Ac at C-2, and require several steps. To date, the most efficient synthesis of peracetylated Neu5Ac2en has been the method presented by C.-H. Wong and co-workers[viii] whereby the peracetylated methyl ester of Neu5Ac is treated with PPh_3HBr in acetonitrile, yielding **1** in 96%.

Despite the presence of these established procedures, we believed the synthesis of **1** could be easily achieved by a thermally induced β-elimination of the C-2 acetate of peracetylated Neu5Ac. We hypothesized that under thermal conditions, the acetate alone could be a sufficiently good leaving group to undergo elimination. Initial attempts at developing this chemistry produced mixed results: heating the peracetylated acid in pyridine yielded the desired eliminated product, but in low yields and produced mainly decarboxylation and dimer formation.[ix] Efforts to reduce these side products by first protecting the acid as a methyl ester failed, as the methyl ester was not observed to undergo elimination or decarboxylation in solution.

While thermal elimination of the C-2 acetate in peracetylated Neu5Ac proved to be unsuccessful in solution, we questioned whether this transformation could be possible in the gas phase under conditions of flash vacuum pyrolysis (FVP). FVP involves the sublimation of a compound at reduced pressure, followed by quickly heating the vapor to pyrolytic temperatures and then immediately cooling the vapor to cryogenic levels.[x] Under these conditions, the compound to be pyrolyzed is subjected to the high temperatures of the column for a very short period of time (usually on the order of 10^{-2} s) and therefore the occurrence of side reactions and degradation is minimized. FVP has seen a number of synthetic applications,[xi] including the syn-elimination of acetates,[xii] however these have mostly been limited to aromatic and low molecular weigh substrates. To our knowledge there have been no reported synthetic applications of FVP in carbohydrate systems.

We constructed a homemade flash vacuum pyrolysis apparatus (Figure S1, see supporting information for details) that would allow us to explore the possibility of acetate pyrolysis with sialic acid. Our first attempts at the gas phase elimination of peracetylated Neu5Ac were unsuccessful; the required temperature to volatilize the substrate resulted only in decomposition. However, initial trials using the methyl ester **2** showed small amounts of product formation by crude ^{1}H NMR analysis [confirmed via olefinic C-3 proton of **1** at δ 6.01 (d, J = 3.3 Hz)]. With this promising lead, we set out to optimize the reaction by varying packing material, column length, temperature, pressure, and flow solvent. After numerous trials we settled on an apparatus of column dimensions 21 cm × 1.8 mm (i.d.) packed with 3 mm diameter pyrex beads. Using these parameters, we further optimized the pyrolysis by screening the reaction at a range of temperatures. The ^{1}H NMR spectra of crude FVP product taken at varying temperatures is displayed in figure 2. Note that as the column temperature was raised, the conversion of **2** to **1** increased to a point of near quantitative transformation (410 °C), yet as the temperature increased further, side product formation was observed and eventually the temperature reached a point where the compound simply decomposed. Interestingly, at temperatures above 420 °C we began to see formation of the

4,5-fused-dihydro-oxazole **3**, a precursor used to prepare Neu5Ac derivatives functionalized at the C-4 position.[xiii]

We found 410 °C to be the optimal temperature for the elimination with our apparatus, yielding nearly quantitative product without the need for workup or purification. Further optimization in terms of mass recovery and overall yield was examined by varying the carrier solvent, and substrate concentration. We found that the total % conversion from **2** → **1** (as analyzed by crude ^{1}H NMR) to be mostly independent of carrier solvent, and we were able to successfully carry out the pyrolysis using benzene, acetone, methanol, acetonitrile, and dichloromethane with similar product purity recovered. However, in terms of overall mass recovery, dichloromethane produced the highest yields. We attribute this to its low boiling point and heat of vaporization which, out of all the solvents screened, cooled the column the least, allowing for precise control of the temperature and high product recovery. However, we believe this observation to be a consequence of our crude apparatus, and feel that an industrially designed device could carry out this method using any number of solvents.

With our conditions fully optimized, we have been able to achieve repeatable yields of 93%.[xiv] We have used this method to prepare upwards of 1 g of the eliminated product **1** in high purity without trouble.

The clean conversion of this reaction suggested an inherent reactivity of the C-2 acetate, and the fact that this reaction takes place in the vapor phase, without the aid of a solvent or base catalyst suggested to us that the transformation proceeds via an intramolecular syn-elimination (scheme 1).[xv]

In an effort to determine the feasibility of our proposed mechanism under the conditions of flash vacuum pyrolysis we employed density functional theory calculations on a model tetrahydropyran system (Figure 3). By visual inspection of the optimized geometry (B3LYP/6-31g(d))[xvi] of the model substrate it is clear that the constrained ring geometry places the C-2 acetate oxygen in close proximity to the C-3 equatorial proton (2.53 Å) but out of reach of the C-3 axial proton. Transition state calculations revealed the desired transition state shown in Figure 3, with a $\Delta G^{\ddagger}$ of 29.26 kcal/mol. Correlating this energy barrier with the temperature of the reaction (410 °C) we can make a rough estimation of the the $t^{1/2}$ of the reaction from eq. 1 and 2, which we have calculated to be 1.42×10^{-3} s.

$$k = \frac{K_B T}{h} e^{\frac{-\Delta G}{RT}} \quad (1)$$

$$t^{1/2} = -\ln(0.5)/k \quad (2)$$

Considering the short time compounds spend in the hot zone during FVP, we find this calculated value to be in agreement with our experimental observations, and these computational results support our proposed mechanism. Transition states of other potential mechanisms were explored including a stepwise E1 elimination, in which the intermediate

cation would be stabilized by oxonium formation. In this mechanism, deprotonation could likely occur at either the axial or equatorial C-3 proton. However, attempts at finding a reasonable transition state for this mechanism failed, resulting only in transition state structures that closely resembled our initial concerted mechanism.

To further investigate the mechanism of this transformation, we prepared the C-3 deuterated substrate **4** (Scheme 2). The base catalyzed H → D exchange at C-3 of Neu5Ac proceeds via a ring opening/tautomerization sequence, and shows higher selectivity for exchange at the axial position,[xvii] presumably due to the extended chirality of the carbohydrate motif. After determining ratio of deuterated isomers of **4** (4 possible, 3 found) by ^{1}H NMR and HRMS, the isotopically labeled substrate was subjected to FVP conditions, and the recovered product purified by HPLC. Analysis of the purified mixture of **5** and **1** revealed a ratio of 20: 1 respectively.[xviii] This corresponds to nearly complete selective elimination of the C-3 equatorial proton, and is in agreement with our proposed syn-elimination mechanism. However, we feel this evidence does not necessarily disprove the stepwise E1 mechanism, and we have not ruled out tight ion pairing between the charged intermediates. Even so, this pathway could be considered more or less a stepwise version of the syn-elimination.

In conclusion, we have developed a reagent-free method for the preparation of Neu5Ac2en by flash vacuum pyrolysis. We believe the method described herein to be the most efficient preparation of peracetylated Neu5Ac2en reported to date; in terms of yield, solvent and reagent cost, time, and waste. Furthermore, we feel that this chemistry could easily be scaled to an industrial level as a flow-like process representing a relatively green synthesis, as it delivers the desired product in high yields without need for reagents or purification. Given the current increased demand for Relenza as a prevention for potential influenza pandemics, this method has potential application toward the commercial production of the drug.

Experimental section

General procedure for flash vacuum pyrolysis

The apparatus (shown in figure S1), consisting of a 21 × 1.8 cm (i.d.) quartz column packed with 3 mm diameter pyrex beads was allowed to heat to the desired temperature in open atmosphere. The apparatus was then opened to a belt driven bench-top high-vac, with pressure monitored by digital manometer. A continuous flow of dry argon was introduced via a 21 gauge needle connected to an argon manifold equipped at one end to a bubble trap. The apparatus was flushed with argon for 20 minutes before an injection was made. The sample to be pyrolyzed was dissolved in the degassed carrier solvent at concentrations ranging from 5 to 200 mg per ml. The dissolved sample was then injected dropwise into the injection flask where it was quickly pulled into the hot column, pyrolyzed, and condensed in the collection flask cooled by liquid N_2. After all the sample solution had been injected, the apparatus was allowed to sit for 2 minutes before the vacuum was cut off and the collection flask removed. The pressure during the pyrolysis was kept between 60 and 200 mBarr.

4,7,8,9-Penta-*O*-acetyl-2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid methyl ester (1)—2 (200 mg, 0.38 mmol) was dissolved in 2 ml of degassed dichloromethane. The solution was injected drop wise into the injection flask of the FVP apparatus, with a column

temperature of 420 °C and a pressure of 65 – 90 mBar. After all of the solution had been injected an additional 0.5 ml of degassed dichloromethane was injected to wash the injection line. The apparatus was then allowed to sit undisturbed for 2 minutes before being pressurized with argon to atmospheric pressure. The collection flask was then removed and allowed to warm to rt. the collected solution was concentrated to yield **1** as yellow oil (170 mg, 95%). ^{1}H and ^{13}C NMR spectra were consistent with literature.[5,xix]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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xiv. This yield reflects the weight of pure recovered product.

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xvi. All calculations were performed using the *GAUSSIAN03W* suite of programs. Geometries and energies reported herein are from B3LYP/6-31g(d) optimizations, characterized by frequency analysis. Reported energies include zero point energy corrections, unscaled. Free energies were calculated at 410 °C, the optimized FVP temperature. See supporting information for more details including coordinates for all calculated structures and full *GAUSSIAN 03* references.

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xviii. Ratio of **5** : **1** determined by ^{1}H NMR integration ratio of C-3 proton at 6.01 ppm relative to other peaks in the spectrum.

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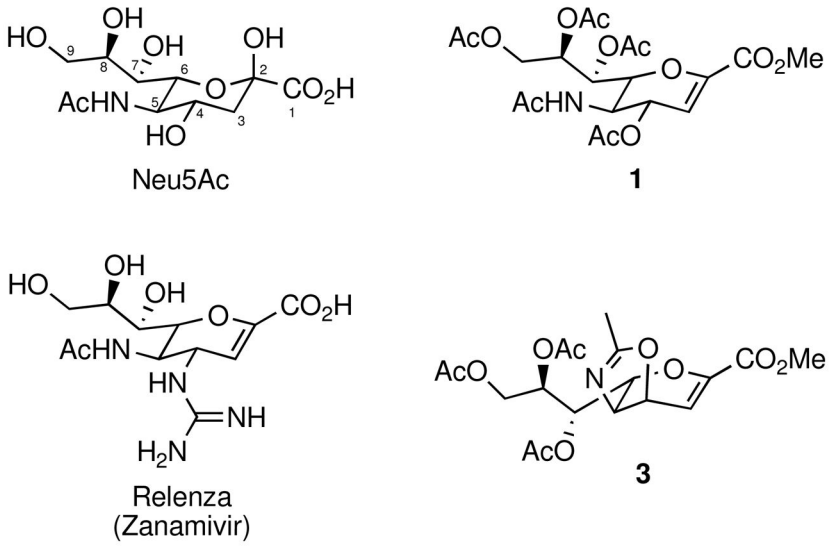


Figure 1.
Structures of Neu5Ac and related derivatives, **1**, **3**, and Relenza

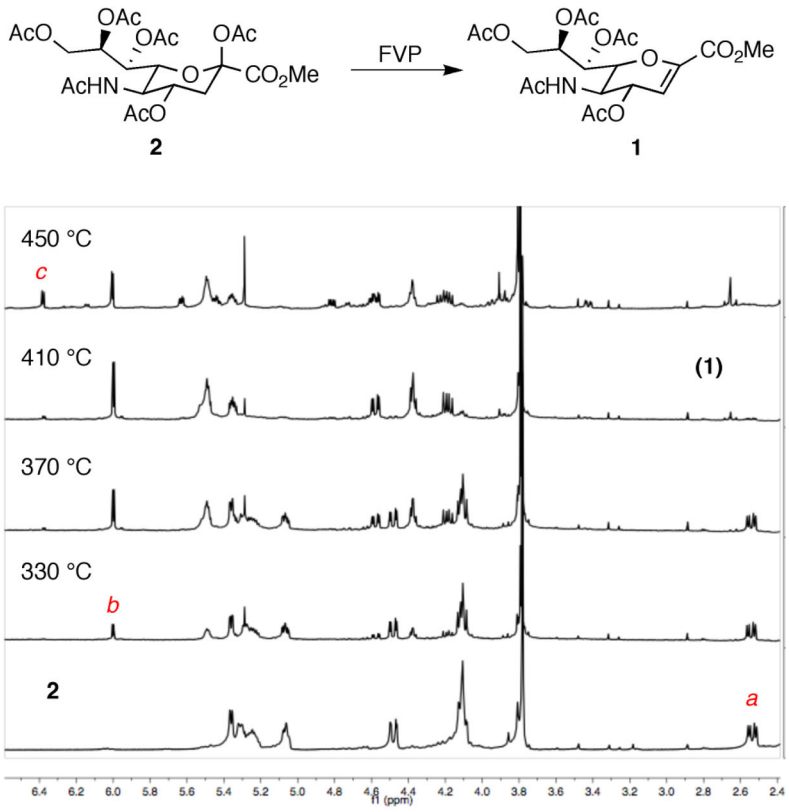


Figure 2.

^{1}H NMR spectra (δ 6.6 – 2.4) of **2** and crude FVP products pyrolyzed at varying temperatures. Characteristic signals a, b, and c correspond to H-3$_{eq}$ of **2**, H-3 of **1**, and H-3 of **3** respectively. All spectra were recorded at 400 MHz in $CDCl_3$ and referenced to residual $CHCl_3$ at δ 7.26. See supporting information for detailed experimental procedures.

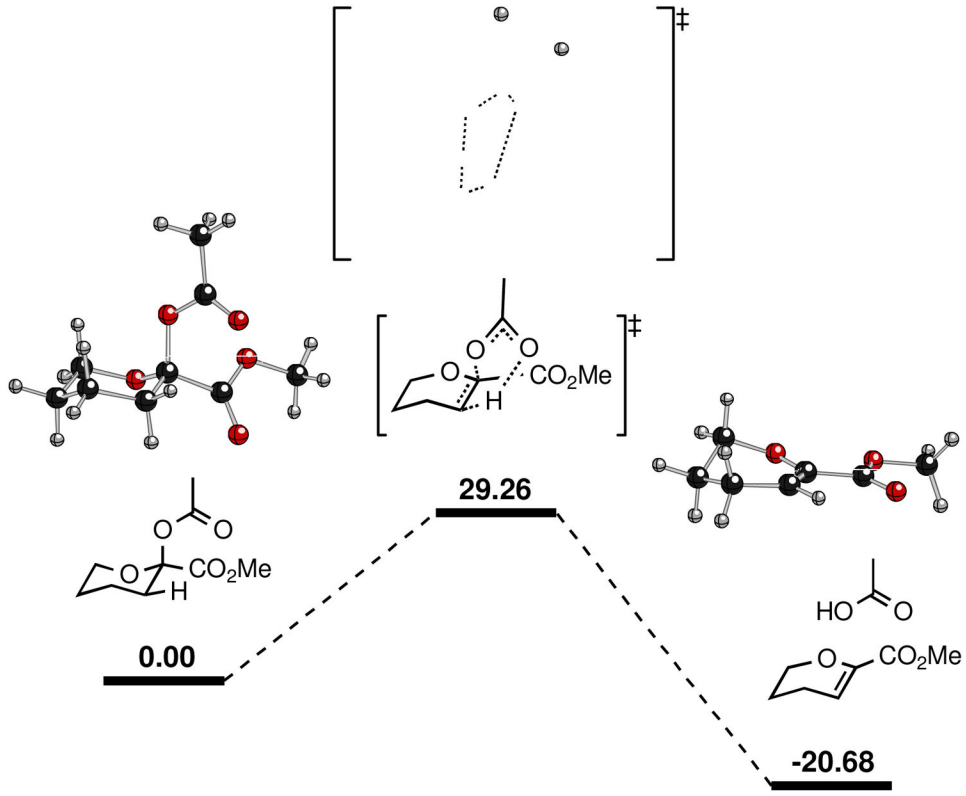
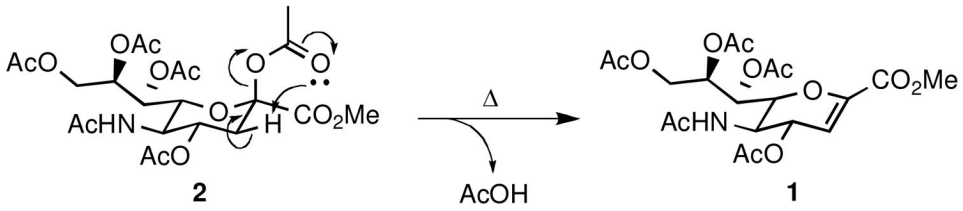
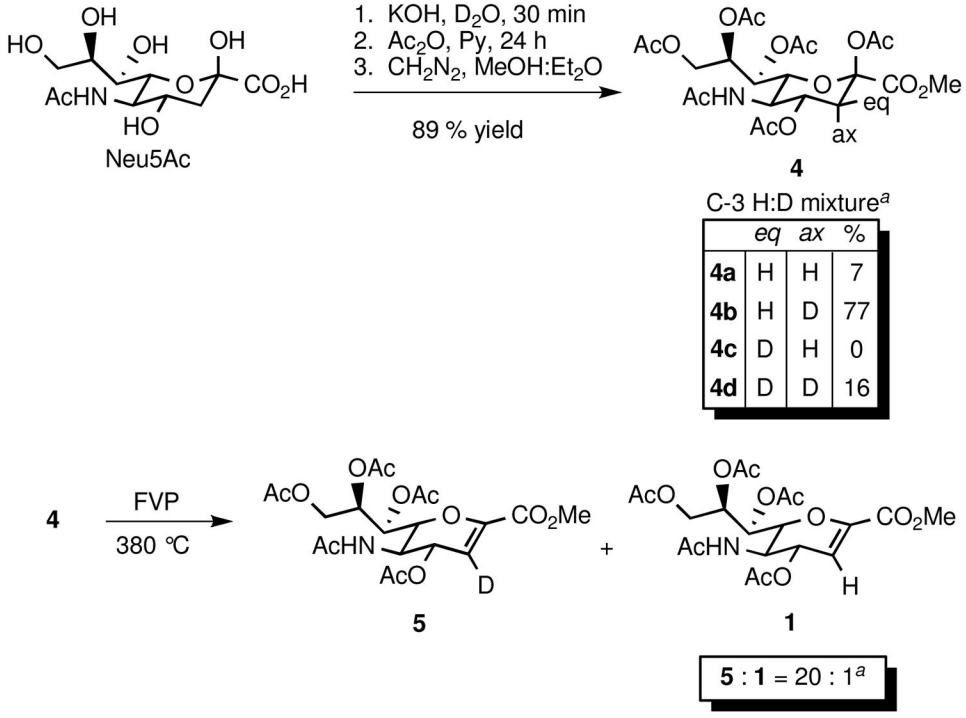


Figure 3.

Relative energy diagram of intramolecular syn-elimination of model tetrahydropyran system. Numbers in bold represent energies (B3LYP/6-31g(d)) in kcal/mol relative to the first structure.



Scheme 1.
Proposed intramolecular syn-elimination



Scheme 2.

Synthesis of ^{2}D labeled substrate and resulting mechanistic study

[a] Ratio determined by ^{1}H NMR integration and HRMS of purified products.