Shedding Light on Heavy Molecules, One by One.

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Introduction.

One of the major motivations for research employing molecular beams is the capability to work with individual molecules under collision free conditions. However, the production of molecular beams has traditionally been limited to molecules with sufficiently high vapor pressures to be effectively seeded without extensive heating. While relatively high temperature molecular beam sources have been used, most larger molecules tend to decompose upon heating. This practical issue has effectively limited the field to small and stable molecules. New techniques have been developed for analytical purposes that produce large molecular ions, such as desorption, MALDI, and ion spray. Of these, only the first can be used to reliably produce intact neutral molecules, which can be combined with a molecular beam. We capitalize on this capability of laser desorption to bring complex neutral molecules into the gas phase without ionization, for fundamental spectroscopic studies. This becomes particularly powerful when the desorbed material is entrained in a supersonic expansion. The cooling in a supersonic jet (a) stabilizes the molecules by reducing their internal energy, (b) makes it possible to carry out spectroscopy, and (c) allows for the study of clusters. The feasibility of this approach has been demonstrated by our own work on polymers and by previous work on small biomolecules. Levy et al. have performed spectroscopy in a supersonic jet of tryptophan, small di- and tri-peptides, and perymidines [1-3]. Lubman et al. have shown jet spectroscopy and mass spectrometry of tyrosine containing dipeptides and similar compounds [4-6]. Schlag et al. have shown that small protected nucleosides and nuleotides can be laser desorbed, jet cooled and multiphoton ionized as well as various amino acids, chlorophylls and porpherins [7-9]. Arikawa et al. studied van der Waals clusters of tryptophan [10]. When extending these techniques to larger molecules we need to address the question whether such systems can be photoionized at all [11; 12]. In what follows we demonstrate that REMPI spectroscopy of molecules with masses of several thousands of Daltons is possible [13; 14], as well as single photon ionization of large hydrocarbons [15]. We also present examples of vibronic spectroscopy of jet cooled small biomolecules and their clusters [16], such as DNA base-pairs.

Experimental approach.

Figure 1 schematically shows the combination of laser desorption with a pulsed molecular beam and a REMPI setup. Material is laser desorbed from a sample probe in front of a pulsed nozzle. The desorption laser is usually a Nd:YAG laser operated at

its fundamental wavelength of 1064 nm. At this wavelength one does not expect photochemical interaction with any of the materials, which we desorb. We have, however, also in many cases successfully used the second and fourth harmonic as well as a 248 nm KrF laser for desorption. Laser desorption involves heating of the substrate, rather than the adsorbate. Therefore it is typically desirable to match the wavelength of the desorbing light with the absorption characteristics of the substrate, while avoiding overlap with the absorption spectrum of the adsorbate. We routinely use graphite as a substrate, although we have also successfully used metal substrates. Typical laser fluences are of the order of 1mJ/cm2. or less, which is significantly less than the fluences normally used for ablation. The laser is focused to a spot of the order of 0.5 mm diameter within 2 mm in front of the nozzle. This is important because in a supersonic expansion most of the cooling takes places close to the nozzle by collisions with the drive gas along a distance of about 10 nozzle diameters. The nozzle consists of a pulsed valve with a nozzle diameter of 1 mm. We usually operate with Ar as a drive gas at a backing pressure of about 5 atmospheres. In earlier work we have optimized the geometry for effective entrainment by mapping entrained perylene with laser induced fluorescence [17; 18]. In that work we found that it is possible to entrain a portion of the desorbed material on the axis of the supersonic beam, such that the ionizing laser downstream can interact with a fraction of about 10-5 of the desorbed material.

Downstream ionization lasers intersect the beam inside the source region of a reflectron time of flight (TOF) mass spectrometer (Jordan Co.). Excimer lasers and a dye laser, used for two photon ionization, intersect the beam at right angles. A vacuum ultraviolet (VUV) beam, used for single photon ionization, is aligned colinearly with the gas beam.

REMPI of polymers.

Figure 2 shows two photon ionization mass spectra of laser desorbed and jet cooled polymers of the form:

$A\text{-}(R)_n\text{-}E$ (A1)

with repeat unit R, a non functional end group E and a functional end croup A, consisting of aromatic ester as follows:

$R = -[CF_2]_3\text{-}O\text{-}$,

$A = -CF_2\text{-}(CO)\text{-}O\text{-}CH_2CH_2\text{-}O\text{-}C_6H_5$,

$E = -O\text{-}CF_2CF_3$.

Bifunctional polymers have the form:

$A\text{-}O\text{-}(R_3)_n\text{-}A$. (A2)

The monofunctional material is commercially available under the brand name Demnum-SP®. All major peaks correspond to parent ions and are spaced apart by 166 Da, the mass of a repeat unit. We have obtained parent molecular peaks from polymers with n = 5 (m/z = 1114 Da) to n = 40 (m/z = 6924 Da). The averages of the molecular weight distributions are consistent with NMR as well as with size exclusion chromatography, which is important for the following reason.

There is current interest in possible fundamental limitations on the size of molecules that can be efficiently ionized [11; 12]. Schlag et al. have proposed that the origin of this limitation is related to the number of vibrationally excited levels that are coupled to the ionizing level. For a large molecule there is a very large number of non-ionic, superexcited states that are isoenergetic with the initially excited ionic state. It has been proposed that the loss of electronic energy (needed to eject the electron) to vibrational degrees of freedom as a result of coupling to these states can interfere with ionization. This is the ionization analog to internal conversion in fluorescence experiments.

We find no evidence of a significant decline in ionization efficiency with molecular size in the current results, which include ionization of molecules up to 7000 Dalton. Nor do we find any evidence for delayed ionization.

By varying the wavelength of the two photon ionization laser, while monitoring polymer parent mass peaks, we obtained excitation spectra. We obtained the $S_{0\text{-}0}$ transition of the polymers, which we found to be slightly redshifted with respect to that of the anisole chromophore and conformationally broadened. We found that the REMPI spectra do not change when the polymer is extended beyond just a few repeat units. This indicates that the excitation is local, in the aromatic chromophore, and completely unaffected by the length of the polymer.

We also obtained spectra of polymer dimers A1-A1, which all show a broadening and a characteristic redshift of about 110 cm-1 from the monomer wavelength. Such a shift is characteristic for a van der Waals interaction between two phenyl rings. The A2 spectra exhibit broadening and redshifts remarkably similar to those of the dimer spectra

The mass spectra in figure 2 were obtained from a mixture of A1 and A2 type polymers. Panel (a) is obtained at the resonant wavelength for A1 polymers. Panel (b) is obtained at the resonant wavelength for A1-A1 dimers, which is also the resonant wavelength for A2 polymers. The similarity in the spectra of the dimers of the monofunctional polymers and the isolated, doubly functionalized polymers leads to the conjecture that the chromophore environment in both cases may be similar. One way of achieving this similarity is to form an intramolecular complex in the type A2 polymers that resembles the intermolecular complex in the van der Waals dimer. This would involve similar interactions between pairs of chromophores in each .

If the intramolecular and intermolecular complexes are formed in the same way, as their wavelength spectra suggest we expect that they both involve interaction of the chromophore ends. This scenario has two prerequisites. First, the chains must be flexible enough so that during the jet expansion they can efficiently bend to bring the

chromophores together. In other words, the barriers to internal rotation must be low enough for the polymers to explore many conformations while they are being cooled, since the experiments suggest that the two ends of the molecule find each other with high efficiency. Second, the interaction between chromophores must be strong enough to effectively form the intramolecular complex once the chromophores are brought together.

Combinations of polymer chains of variable flexibility with different chromophores will provide an interesting arena for making predictions based on barriers to internal rotation and binding energy. Gas phase spectroscopic measurements, similar to those reported here, of the interaction between chromophores at remote positions on polymer chains may be excellent tests of those predictions. It may be possible to gauge the internal barrier heights with a sequence of chromophores of ranging dimer strength.

REMPI of biomolecules

Peptides.

An example of REMPI spectroscopy of laser desorbed, jet cooled dipeptides appears in figure 3. We compare the spectrum of tyrosine with two dipeptides that contain tyrosine as a chromophore, Tyr-Ala and Ala-Tyr. We have recorded a much larger wavelength range and many more peptide combinations, but here we merely show the origin region of representative examples. The structure in the spectra is due multiple conformations and possibly to very low energy vibrations. The possible conformations for tyrosine and its derivatives have been discussed by several authors [19-23]. Some tentative assignments have been proposed based on several assumptions and comparisons with derivative spectra. When comparing with the dipeptides it appears that either the number of conformations increases drastically, or there are sequences of low energy vibrations. The former interpretation seems less likely because these multiple peaks do not occur for dipeptides in which we replaced tyrosine with phenylalanine. The only difference between these molecules is the hydroxyl group which is present in Tyr and absent in Phe. Another remarkable feature is the group of peaks to the red in Ala-Tyr, which may be due to an interaction of the amino terminus with the –OH group in the tyrosine ring, similar to the intramolecular exciplexes observed in tryptophan << - Not Found>>.

In addition to structural information that can be obtained from these spectra, we notice the tremendous isomeric distinction that can be obtained by wavelength dispersion in addition to mass spectral detection.

Nucleotides

The study of spectroscopic properties of DNA bases is of fundamental importance. Both vibrational and electronic spectroscopy can help elucidate issues of structure, bonding, and reactivity. UV spectroscopy, especially in the 300 nm region, is also

crucial for understanding the photophysics and photochemistry of genetic material. In order to facilitate investigation of complex oligonucleotides, we need detailed information on the basic building blocks, single nucleotides and nucleosides. Most work to date on those molecules has been in solution and in the solid state.

There have been many theoretical studies of vibrational and electronic states of DNA bases [24-26]. These are complicated by multiple lone pair electrons, limited symmetry, and possible tautomerism. Experimentally there have been many reports of absorption spectroscopy [27; 28], IR data [29; 30], and Raman spectroscopy [31]. Samples have been in the form of vapour, solutions, polycrystalline material, single crystals, and cold matrices. Gas phase studies can offer the advantage of eliminating intermolecular interactions.

Figure 4 shows the REMPI spectrum of jet cooled guanine, substituted guanines, and guanasine. A detailed computation of S_1 state vibrations is under way. Absent that, we can make initial assignments by comparison with ground state calculations and with matrix IR data. It is interesting to point out the difference between methyl substitution in the 1 and the 9 position. The latter shows a much larger spectral shift, suggesting that the electron pair associated with the 9 nitrogen is more important than for the S_1 state than that on the 1 nitrogen. We note that the sugar in guanasine is attached in the 9 position. The low energy vibrations in the substituted guanines can be understood as vibrations, such as torsion, between the chromophore and the substituent.

DNA base pairs.

In order to form isolated base pairs in the gas phase we laser desorb a mixture of neat guanine (G) and cytosine (C) from a graphite surface, followed by entrainment in the supersonic expansion of argon. Figure 5 shows the REMPI spectrum of the GC dimer. For comparison we also show the spectra of the GG dimer and of the G monomer, which we recorded simultaneously in the same experiment. The spectra are offset such that the origins (0-0 transitions) are shown at the same position. Actually the GC origin is blue-shifted by 435 cm-1 with respect to the G origin, which is at 32878 cm-1. The GG origin is blue-shifted with respect to that of G by 224 cm-1. Neither the G nor the C chromophore of the dimers absorbs in the first 200 wavenumbers above the origin of the complex. The guanine spectrum exhibits its first significant vibronic activity at 235 cm-1. Cytosine absorbs altogether much further to the blue at about 36000 cm-1, similar to uracil and thymine [3]. Therefore, when we excite the guanine chromophore in the GG and GC complexes, we can observe hydrogen bond vibrations built on the 0-0 transition *without any interference*. Indeed all spectral lines in the first two hundred wavenumbers of these spectra can be understood as hydrogen bond vibrations.

When we compare our experimental frequencies with the ab-initio calculated values of Florian et al.Florian 1995 421}, Shishkin et al.Hobza 1999 15}, and Santamaria et al.Santamaria 1999 511}, we find a remarkable agreement between experiment and theory. However, we should note that this represents a comparison between excited

state measurements and ground state calculations. A final assignment has to be postponed until reliable S1 state calculations, preferably on the CASSF level, are available.

Ab initio calculations of the GG structure at different levels of theory agree that the symmetric structure displayed in figure 5 is the most stable one [32; 33]. When both monomer parts in the dimer are completely identical, only one electronic spectrum is expected. We see no hint of band exciton splittings or a second electronic band system in the investigated spectral range. We therefore conclude that GG has the double hydrogen bonded highly symmetrical structure depicted in figure 5.

We desorbed from an equimolar mixture of guanine, adenine, cytosine, thymine, and uracil and found mass peaks for all combinations of G with the other bases at high ionization laser power under non-resonant conditions. We did not, however, observe any vibronic resonances for any of the possible complexes besides those of GG and GC in the investigated spectral range. Ab initio calculations at different levels of theory agree that the GC Watson-Crick base pare with three nearly linear hydrogen bonds and the symmetrically hydrogen bonded GG base pair are by far the most stable base pairs [34].

Summary

By combining laser desorption and entrainment in a supersonic beam, we can study large and fragile molecules in the gas phase. Such molecules have vary low vapor pressures and would normally decompose when heated. By entraining laser desorbed neutral molecules in a supersonic expansion, we can create a beam of large cooled molecules. We demonstrated REMPI spectroscopy of molecules with masses of several thousands of Daltons. We applied this capability to study internal and external dimer formation in polymer. We presented examples of vibronic spectroscopy of jet cooled small biomolecules and their clusters, such as dipeptides, DNA base and DNA base-pairs.

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Figure 1 Schematic diagram of the experimental setup.

Figure 2 Laser desorption jet cooling mass spectra of perfluoropolyether, by two photon ionization of aromatic end groups.. Panel (a) at the resonant wavelength for A1 polymers. Panel (b) at the resonant wavelength for A1-A1 dimers, which is also the resonant wavelength for A2 polymers

Figure 3 REMPI spectra of laser desorbed tyr, tyr-ala, and ala-tyr.

Figure 4 REMPI spectra of guanine, 1-methyl-guanine, 9-methyl-guanine, and guanaside.

Figure 5 REMPI spectrum of guanine (G) and its dimers with guanine (GG) and cytosine (GC). The spectra are offset such that the origins (0-0 transitions) are shown at the same position for all three species.

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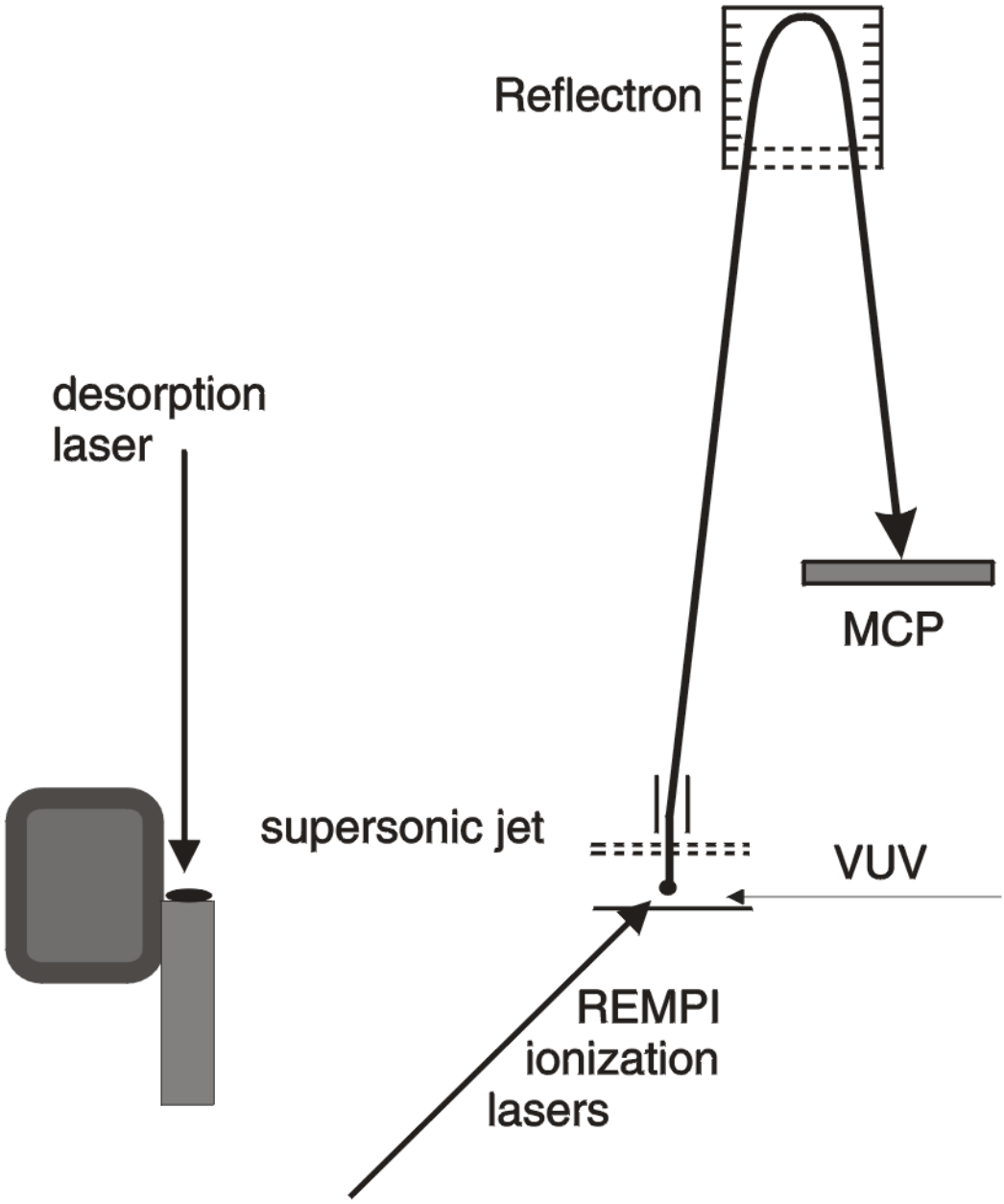
Reflectron
desorption
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ionization
lasers

FIGURE 1

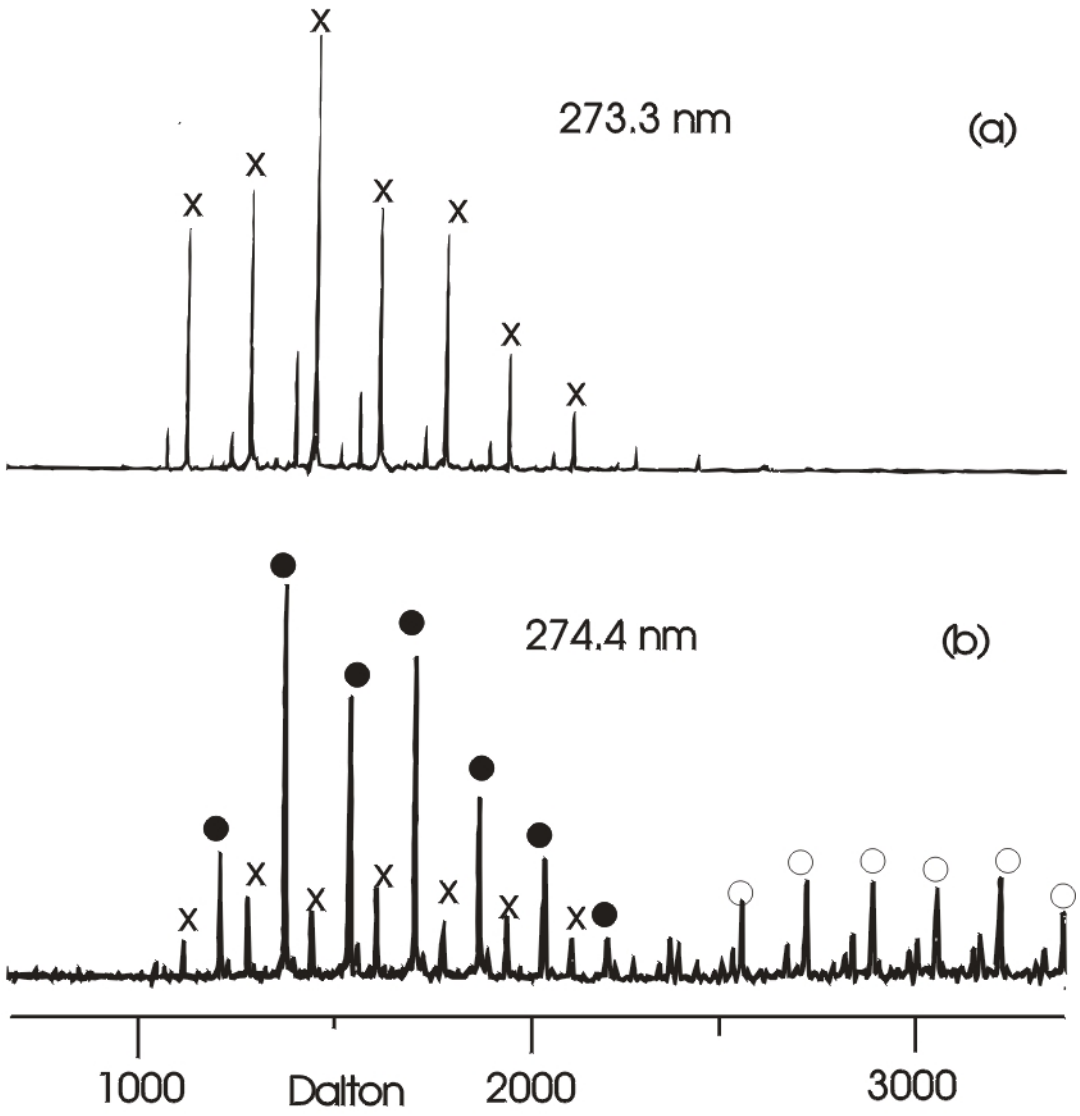

FIGURE 2.

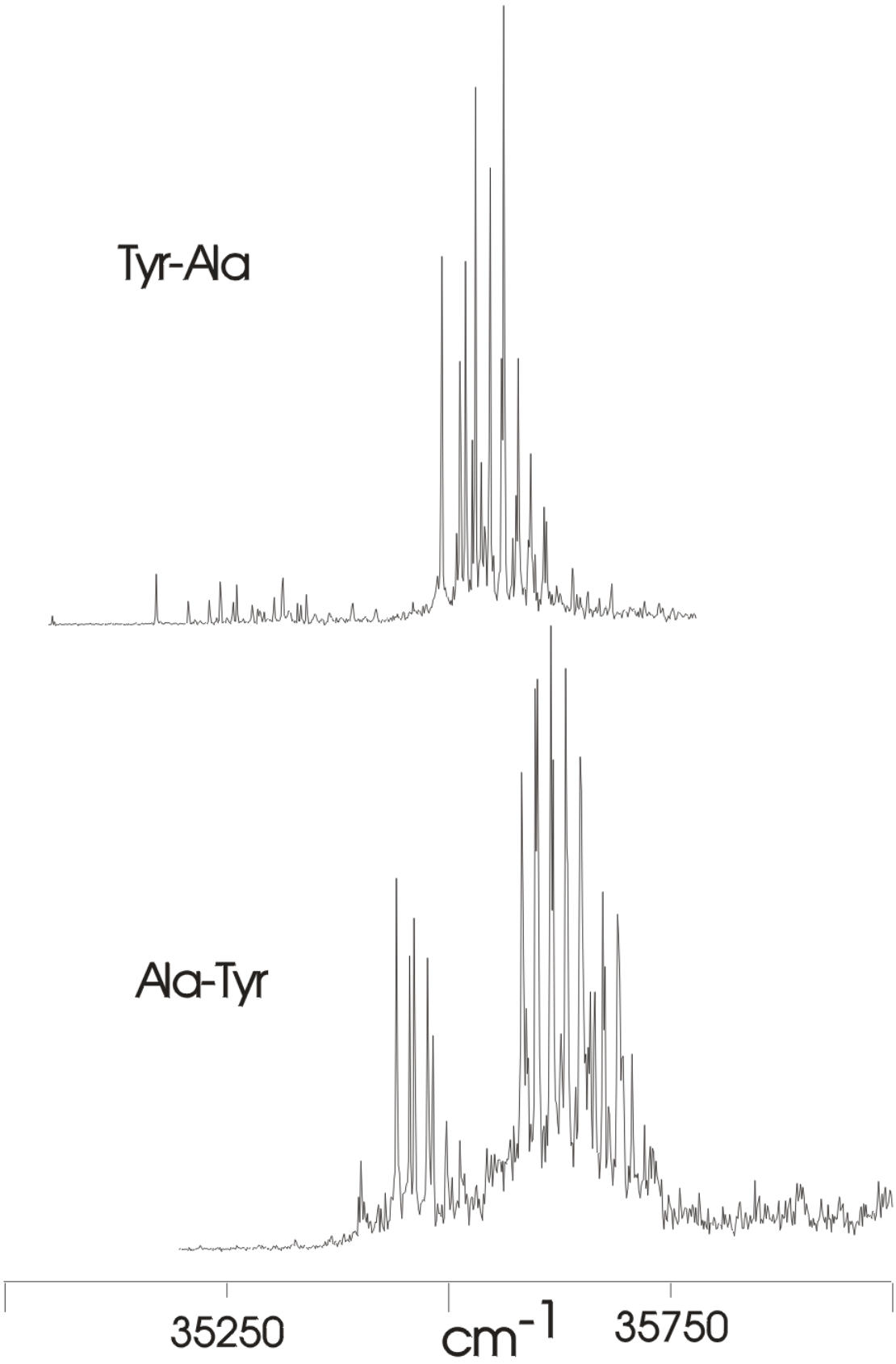

FIGURE 3

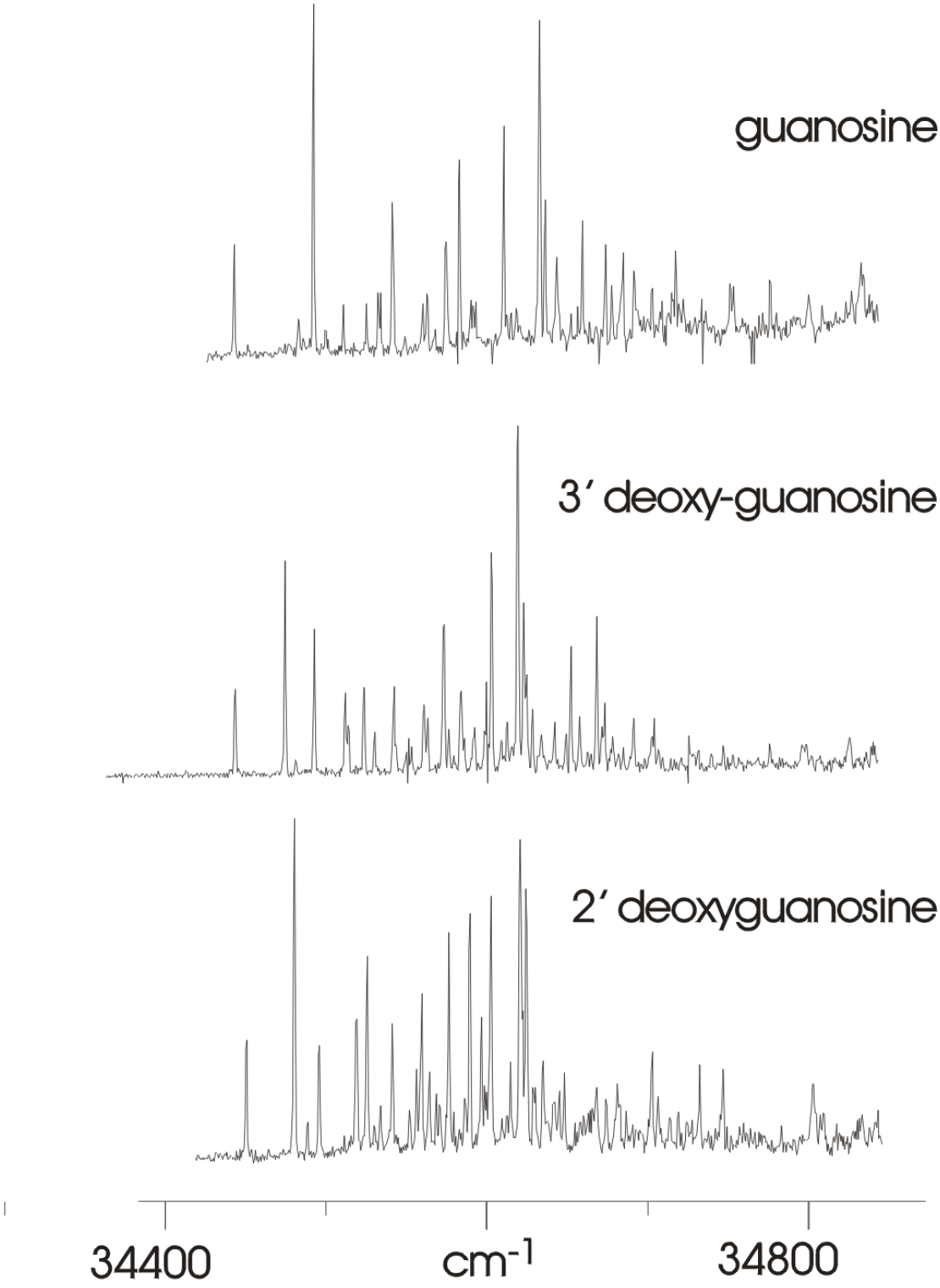
guanosine
3′ deoxy-guanosine
2′ deoxyguanosine
34400
cm-1
34800

FIGURE 4.

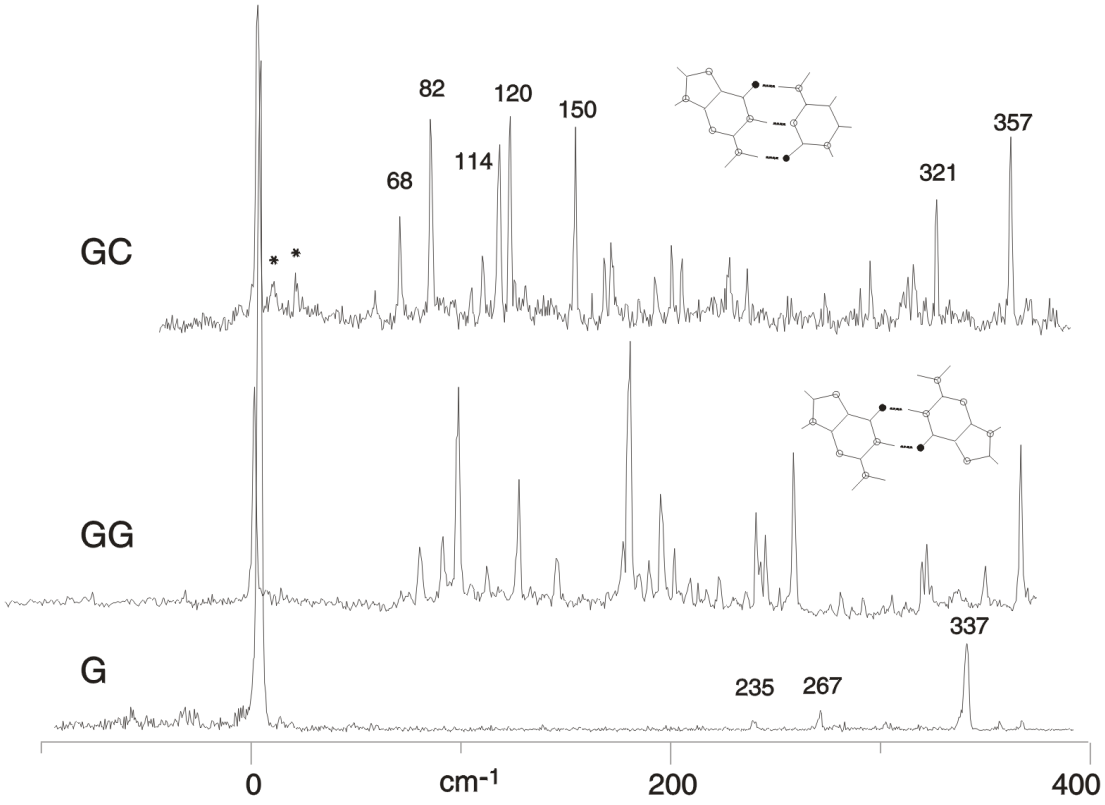

FIGURE 5.