^{13}C to ^{19}F Dipolar Couplings as an NMR Structure Probe[1]

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The homonuclear (^1H to ^1H) dipolar-coupled NMR experiment has been used to determine the structures of several small molecules dissolved in liquid crystals. However, the spectra of these experiments are difficult to duplicate by calculation if there are more than nine strongly coupled hydrogens. To overcome this severe limitation of a potentially useful structural technique, the dipolar couplings between ^{19}F and natural abundance ^{13}C were used. The reduction in coupled nuclei greatly simplifies the calculations and allows the theoretical refinement of any dipolar-coupled spectrum to a structure. The technique is demonstrated by application to *p*-difluorobenzene. As the introduction of a single fluorine is synthetically difficult, this paper shows how the more easily introduced trifluoromethyl moiety as the acetyl and the acetate groups could be used as the source of ^{19}F. The utilization of these functionalities is demonstrated in the structural refinements of 3-(trifluoroacetyl)camphor and cyclohexanol trifluoroacetate.

With the introduction of the rare-earth chelates to NMR spectroscopy, it was at first thought that the problem of structure determinations in solutions was solved. As the lanthanide induced shift (LIS) method was explored and developed, limitations to the technique were encountered. For all practical purposes the LIS technique was applicable only to relatively rigid molecules which contain only one strong Lewis base site.[3] This limitation made it desirable that another, generally applicable, NMR structure determination teqhnique be explored. One possible technique is the dipolar-coupling experiment.

The magnitude of the dipole–dipole nuclear spin coupling constant is as shown in eq 1.[4] θ_{ij} is the angle made

$$D_{ij} = [-L_{ij}/4\pi^2 r_{ij}^3][\tfrac{1}{2}(3\cos^2\theta_{ij} - 1)] \qquad (1)$$

by the vector between the two coupled nuclei (r_{ij}) and the external magnetic field axis, the 3 cos θ_{ij} term of eq 1 can be expanded to equal

$$\frac{1}{2}(1 - 3\cos^2\gamma)(1 - 3\cos^2\phi)S_{ij}$$

where γ is the angle between r_{ij} and the major molecule rotation axis, ϕ is the angle between the major and secondary axis of molecular reorientation, and γ' is the angle

$$S_{ij} = \frac{3}{2}(\cos^2\gamma' - \tfrac{1}{2})$$

between the molecular axis and the direction of the preferred molecular orientation in the magnetic field.[4]

In a nonviscous solvent the solute molecule (substrate) experiences essentially unrestricted isotropic motion. In this case the observed NMR spectrum is a time-averaged one and $D_{ij} = 0$. If the motion of the molecule is restricted, dipolar couplings are observed and can be extracted and assigned and the structure of the substrate refined. This condition of restricted motion is met in solids, but the observed dipolar couplings are not only between nuclei of the same molecule but also between nuclei of adjacent molecules. This results in a wealth of spectral lines that may not be resolved. In order to eliminate these intermolecular couplings, yet retain the intramolecular couplings, it is necessary to allow the substrate to diffuse essentially independently from its neighbors but retain the same orientation with respect to the external magnetic field. These conditions are met by using a nematic liquid crystal or a laminal lipid bilayer system as a solvent for the substrate.

Nematic liquid crystals can exist in three morphologically distinct phases which are temperature dependent. The phase of interest for NMR structural studies is the nematic one which may be described as an ordered liquid. The crystals line up in the NMR tube in response to the external magnetic field, and the substrate molecules dissolved in the liquid crystals are oriented between the lines of the crystals. This orientation restricts the rotational motion of the substrate but allows for translational motion along the axis of the liquid crystal.[5] This unique situation eliminates the intermolecular couplings but retains the intramolecular structural couplings. For example, pyridine in *p*-ethoxybenzylidene-*p*-*n*-butylaniline (EBBA) is observed to contain five nonequivalent hydrogens.[5] Since all of the hydrogens are strongly coupled to each other, they must be calculated together as a unit. The lack of molecular symmetry requires a Hamiltonian matrix which because of its large size can just be handled by our computer system and for all practical purposes is the largest compound whose structure can be determined by this method. To extend the range of compounds whose structures can be determined by this NMR method, the possibility of heteronuclear coupling to natural abundance ^{13}C was investigated.

The effect of using an NMR-active nucleus other than hydrogen to couple with natural abundance ^{13}C can greatly simplify the calculations. Instead of being forced to calculate all of the observed nuclei at the same time, the molecule can be factored into n number of separate 2×2 Hamiltonian matrices. This factoring can be done because there is statistically only one ^{13}C per substrate molecule and effectively no carbon–carbon couplings. Each unique carbon position can be calculated independently of all others. The entire molecule can then be reassembled by the addition of the independent calculations. Such factoring eliminates all of the previously described computational problems of the technique. Fluorine was chosen as a heteronucleus because it occurs as virtually 100% of the NMR-active isotope, it has the third highest sensitivity relative to protons, and it exhibits very strong dipolar

(1) The idea of using ^{13}C to ^{19}F dipolar couplings rather than ^1H to ^1H dipolar couplings for structure determinations was originally suggested by Professor J. D. Roberts of the California Institute of Technology in a private communication to W. H. Bearden.

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(3) Bearden, W. H. Ph.D. Dissertation, University of Houston, 1975.

(4) Buckingham, A. D.; Dunn, M. B. *Mol. Phys.* **1969**, *19*, 721.

(5) Diehl, P.; Khetrapal, Z. L.; Kellerhals, H. P. *Mol. Phys.* **1968**, *15*, 333.

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couplings to ^{13}C. The latter virtue of ^{19}F becomes very important when complex molecular structures are attempted, as the large dipolar couplings increase the frequency range over which the spectrum is spread.

The initial experiment using ^{19}F coupled to ^{13}C was run with *p*-difluorobenzene as the substrate. This seemingly trivial substrate was used because of its well-established structure and its availability. The latter criteria brings up one of the major drawbacks to using ^{19}F as the heteroatom—that it is difficult to introduce synthetically into a variety of potential subject molecules. To overcome this problem, the trifluoromethyl moiety was used in the forms of acetyl and acetate as the source of the heteroatom ^{19}F. Synthetically these functionalities are much easier to introduce than a single ^{19}F, but the potential experimental problems of using trifluoromethyl are somewhat ominous. In the extreme case, the three fluorines of the trifluoromethyl may no longer be magnetically equivalent, which would produce a very complex spectrum. In addition to this potentially fatal problem, there is also the rotation of acetate or acetyl with respect to the rest of the molecule. If the rotation is stopped, then the calculations are essentially the same as a single fluorine. If the rotation is slow on the NMR time scale, then the spectrum will be composed of an infinte set of lines, each set arising from the different orientation of the trifluoromethyl moiety with respect to the external magnetic field—clearly an untenable situation. A third possibility is that the trifluoromethyl moiety is rapidly reorienting in the NMR time frame. In this case the calculations are the same as a single source of ^{19}F with the exception that an averaged location of the trifluoromethyl must be used. To resolve these questions 3-(trifluoroacetyl)camphor and cyclohexyl trifluoroacetate were run in the nematic liquid crystal EBBA.

Experimental Section

The dipolar-coupled NMR spectra were obtained in the following manner. The nematic phase sample of the appropriate substrate in the liquid crystal (EBBA) was prepared by adding the substrate dropwise into a 10-mm NMR tube containing the nematic EBBA. The maximum concentration of substrate that could be added to the EBBA and still maintain the nematic state was 0.15 M. The samples appeared to remain stable for several days if the temperature was maintained at 5.0 ± 0.1 °C.

The sample, complete with a capillary lock source (dimethyl-d_6 sulfoxide), was placed in the thermostated, fixed-frequency probe (22.6 MHz for carbon-13) of the Nico Bruker-90 spectrometer.[6] A standard one-pulse experiment was run on the nonspinning sample by using a 70° pulse angle and a repetition rate of 5 s. After 5000 scans the spectrum was transformed, a blank spectrum of the EBBA run under identical conditions was subtracted, and the resulting difference spectrum was stored for the purpose of block averaging. Up to eight of these different spectra were added and the resulting block-averaged difference spectrum was used for the subsequent analysis.

Because of the uncertainties in the spectra and the historical precedence of pyridine,[5] each of the carbons in the substrate was treated as if it were magnetically nonequivalent for the purpose of computer simulation. The simulation was done with a modified LACOON program LEQUOR.[3] The input parameters for each carbon were chemical shift, *J* coupling, and estimated *D* coupling. The *J* couplings and chemical shifts were obtained from an isotropic phase spectrum of the substrate in EBBA. The *D* couplings were obtained from the nematic phase spectrum by inspection. After the initial calculations for each carbon, line assignments were made and the program was allowed to iterate first on the *D* values for proper line separation and then on the chemical shifts for proper relative location. Again, since each carbon was calculated independently, this technique could be used for any size molecule.

(6) This is a Bruker WH-90 equipped with a Nicolet 293-A pulse programer and Nicolet software.

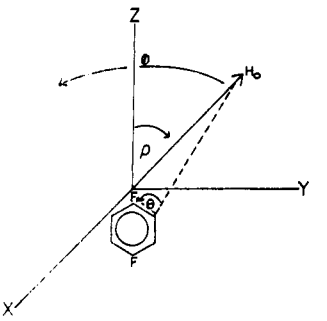


Figure 1. Orientation of *p*-difluorobenzene in the program CD-SDHAPE.

Finally, the lines from all of the carbons in the substrate were plotted and compared to the observed nematic phase spectrum.

The *D* couplings, along with a set of internally referenced *x*, *y*, and *z* coordinates for each carbon of the proposed structure for the substrate, were used as input for another program, CD-SHAPE.[7] This program uses a unit vector to represent the external magnetic field, the position of which is fixed by two angles, ρ and ϕ (see Figure 1). These angles are incremented at a programmed, stepwise rate, and at each position an r_{ij} and angle θ are calculated. These were substituted into eq 1 and a set of D couplings were produced. The calculated couplings were then compared to the experimental couplings by the normalized error analysis formula—Hamilton's *R* factor).[8] The minimum *R* factor is assumed to present the best orientation of the molecule in the magnetic field. From previous experience in using this error analysis, an *R* factor of greater than 10% indicated a poor set of internal coordinates or an improper magnetic field orientation.[3] In those cases where intramolecular reorientation was suspected, the reorienting moiety was rotated about its principal axis at 20° intervals and the calculations were done for each rotation.

The substrates *p*-difluorobenzene and 3-(trifluoroacetyl)camphor were obtained from Aldrich and were used after simple distillation. The liquid crystal used in these experiments was prepared from the condensation of *p*-ethoxybenzaldehyde with *p*-*n*-butylaniline, both obtained from Aldrich.[9]

The substrate cyclohexyl trifluoroacetate was prepared from cyclohexanol and trifluoroacetic anhydride by direct esterification. The workup of this compound was accomplished by fractional distillation under vacuum.

Results and Discussion

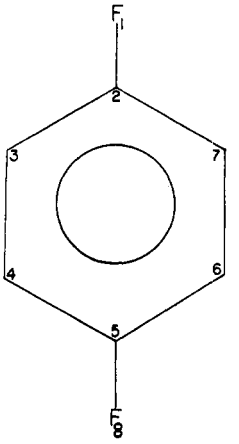
***p*-Difluorobenzene.** The nematic phase spectrum of *p*-difluorobenzene was obtained as described in the Experimental Section. It consisted of a number of broad overlapping resonances. The results of the simulation of this spectrum with LEQUOR are shown in Table I. A comparison of the observed *D* coupling values with those calculated *D* values using Hamilton's *R* factor method yielded a reasonable agreement of 2.2%.[8] The residual error in the simulation is undoubtedly a result of the broad resonance lines in the spectrum. Even though the results for each carbon were calculated independently, the identical calculated couplings of carbons 3 to 7 and 4 to 6 allows the inference that the orientation of the molecule in the magnetic field is such that there exists a plane of symmetry bisecting the fluorine atoms 1 and 8 (a side to side symmetry). In contrast, there does not appear to be any plane of symmetry equating the top to the bottom (see Table I). The results of these calculations would inciate that the substrate was allowed to equilibrate by rotation in only

(7) CD-SHAPE is a modified version of the R. E. Davis program PDIGM which is described: Willcott, M. R. III; Lenkinski, R. E.; Davis, R. E. *J. Am. Chem. Soc.* **1972**, *94*, 1742.

(8) Hamilton, W. C. *Acta. Cryst.* **1965**, *18*, 502.

(9) "Organic Syntheses", Robjohn, N., Ed., Wiley: New York, 1950, Collet. Vol. 4, pp 30–39.

Table I. Observed *D* Values (hertz) from LEQUOR for *p*-Difluorobenzene



D(1,2)	4055.6 ± 12.5
D(1,3)	1307.3 ● 67.5
D(1,4)	226.0 ± 27.1
D(1,5)	185.6 ± 16.5
D(1,6)	226.0 ± 25.0
D(1,7)	1307.0 ± 72.0
D(2,8)	190.6 ± 14.3
D(3,8)	283.0 ± 26.0
D(4,8)	1298.5 ± 29.1
D(5,8)	3833.9 ± 16.5
D(6,8)	1300.6 ± 29.0
D(7,8)	278.0 ± 25.0

R = 2.23%

Table II. Calculated *D* Values (hertz) from CD-SHAPE for *p*-Difluorobenzene

atoms coupled[a]	*D*(calcd)	obsd − calcd[b]
F_1–C_2[c]	4013.8	41.8
F_1–C_3	1281.3	26.0
F_1–C_4	263.7	−37.7
F_1–C_5	190.2	−4.6
F_8–C_2	196.8	−11.2
F_8–C_3	263.7	19.3
F_8–C_4	1181.3	117.2
F_8–C_5	3848.2	−14.3
	R = 1.0%	

[a] See figure in Table I for atom numbering. [b] The calculated values are from Table I. [c] These assignments are the same as Table I.

one mode—around the axis bisecting the two fluorine atoms.

The *D* couplings of Table I were used as input along with the coordinates of each carbon and fluorine atom in the previously described program CD-SHAPE.[7] The results of these calculations are in Table II. As shown in this table the agreement between the theoretical geometry of *p*-difluorobenzene and the geometry produced by the *D* couplings is excellent. As a check on the results from CD-SHAPE, the coordinates of each of the unique carbon atoms were changed by 0.1 Å and the calculations of CD-SHAPE repeated. In every case these changes resulted in a significant difference in the calculated R factor. The confidence interval for rejection of these changes in the carbon atom positions is greater than 99.5% in the worst case.[10] In addition to the statistical method of evaluating these results, the orientation of the molecule in the magnetic field as shown from CD-SHAPE is indicative of the orientation of the nematic liquid crystal in response to the magnetic field. From Figure 2 the long axis of *p*-difluorobenzene is 12° from the magnetic field axis. If this axis of the substrate coincides with the major axis of the nematic liquid crystal, then the orientation of the liquid crystal with respect to the magnetic field is in agreement with predictions based on previous work.[11]

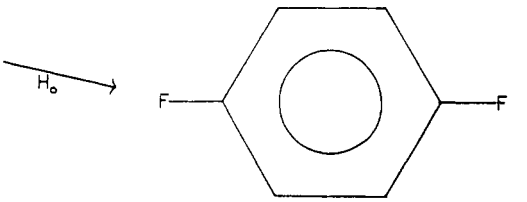


Figure 2. Orientation of *p*-difluorobenzene in the external magnetic field.

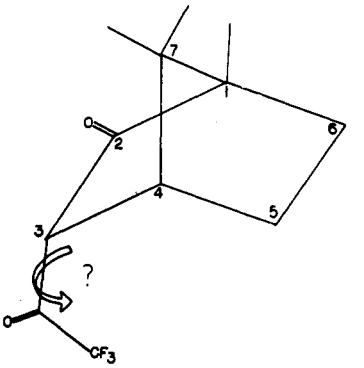


Figure 3. 3-(Trifluoroacetyl)camphor.

The excellent way in which the structure of this molecule refined led us to attempt the use of the more easily introduced trifluoromethyl moiety as the source of ^{19}F.

3-(Trifluoroacetyl)camphor. The major reasons that this substrate was chosen to test the ability of the trifluoromethyl moiety to serve as the source of ^{19}F was its well-known structure, its commercial availability, and its rigidity. On the latter point, as was explained in the introduction, intramolecular reorientation of the substrate may invalidate the use of trifluoromethyl as the source of ^{19}F. This molecule has only two of these modes—rotation about the CF_3—C=O bond and/or about the C3-C=O bond (see Figure 3). Both of these rotational modes, if slow on the NMR time scale, would produce an unresolvable spectrum.

The dipolar-coupled spectrum of this substrate was obtained in the same manner as *p*-difluorobenzene. Gratifyingly, the spectrum consisted of well-resolved lines, narrower and over a wider chemical shift range than those observed for *p*-difluorobenzene. This gross observation immediately eliminated the worst case possibility—a slow reorientation of either of the two rotational modes. In addition, this same observation made a strong case for the rapid time-averaged reorientation about the CF_3—C=O bond as there were insufficient spectral lines for the three fluorines to be anything but magnetically equivalent. The problem of the second rotational mode can easily be handled by using either a static or a time-averaged computational model.

The dipolar-coupling constants and the coordinates of the carbons were obtained as before with the exception of the acetyl carbonyl carbon and the trifluoromethyl group. These two coordinates were obtained by the rotation about the C3—C=O bond in 20° steps. At each rotational step all the carbon coordinates were loaded along with the observed dipolar couplings into CD-SHAPE and the calcu-

(10) Hamilton, W. C. "Statistics in Physical Science"; Roland Press: New York, 1944; pp 157–162.

(11) Nering, J.; Saupe, A. *Mol. Cryst. Liquid Cryst.* **1969**, *8*, 403.

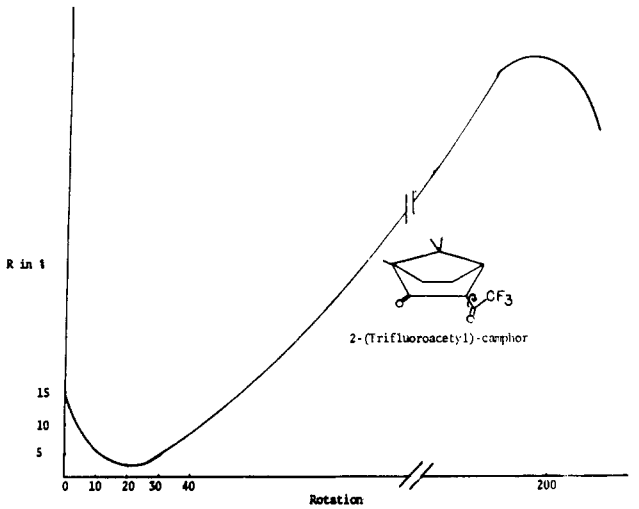


Figure 4. Rotation about C3—O=O vs. %R.

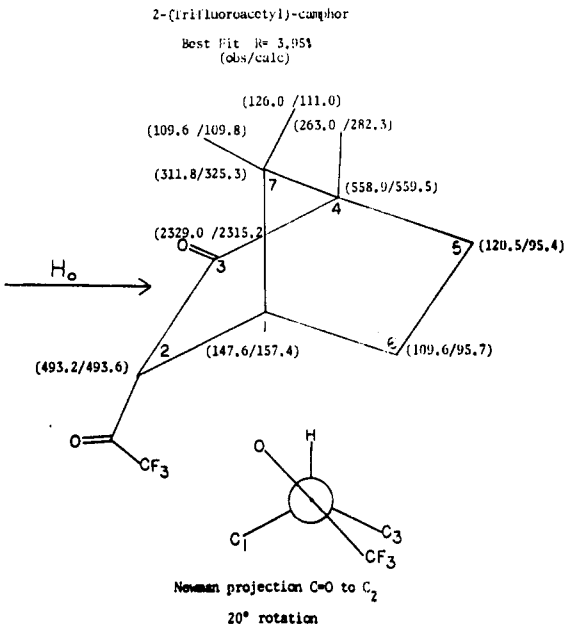


Figure 5. 3-(Trifluoroacetyl)camphor orientated in the magnetic field; D's (obsd/calcd), best fit R = 3.95%.

lations were done as before. To test the time-averaged model, all of the rotational coordinates of the C=O and the trifluoromethyl atoms were arithmetically averaged to obtain the "time-averaged location" of these atoms. The results of the calculation were such that the time-averaged model could be rejected on the 99.999% confidence interval when compared to the best single location model. Figure 4 shows the results of the stepwise rotation of the trifluoroacetyl moiety. The minimum R factor was found to be at the 20° rotation, but we were unable to fix the exact location within the range between 20° and 60° above the 95% confidence interval. The apparent imprecision in the location of the trifluoroacetyl moiety can be rationalized by recalling that the liquid crystal which restricts this rotation is sterically inhibited by the bulk of the molecule from interacting with the relatively smaller portion of the substrate. To show that this is a plausible explanation, the location of each of the carbons was changed in the [2,2,1] portion of the molecule and the CD-SHAPE calculations were repeated. The results were a significant (99.95%) decrease in the R factor of the altered model. If the location of the rigid portion of the substrate can be fixed with this reliability, then the apparent

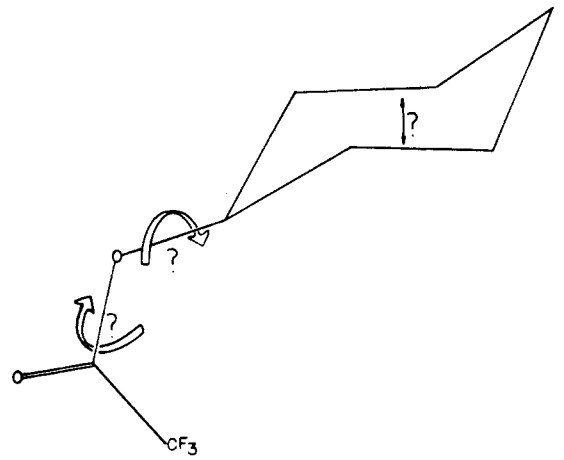


Figure 6. Cyclohexyl trifluoroacetate.

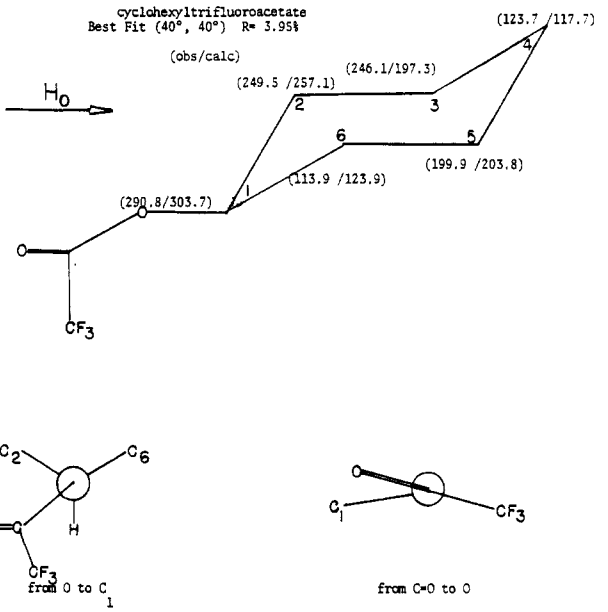


Figure 7. Cyclohexyl trifluoroacetate orientated in the magnetic field; D's (obsd/calcd), best fit R = 3.95%.

movement of the trifluoroacetyl is not an artifact. The location of the trifluoromethyl at the 20° rotation and the orientation of the substrate in the magnetic field are shown in Figure 5. The results shown here were as anticipated. The trifluoroacetyl is pointed away from the bulk of the molecule and the substrate is essentially aligned with the magnetic field.

Cyclohexyl Trifluoroacetate. While the experiments with trifluoroacetyl showed that the trifluoromethyl moiety was a usable source of the ^{19}F, it is not a convenient source of ^{19}F. A more synthetically attainable source would be the trifluoroacetate ester. However, the ester functionality has two modes of rotational freedom, one about the C1–O bond and the second about the O—C=O (see Figure 6). Computationally, this problem is resolved in the same manner as was previously demonstrated. A set of coordinates was generated for the rotation about the C1–O bond. For each one of these sets of coordinates, a subset of coordinates involving the rotation about the O—C=O was generated and used for the CD-SHAPE calculations. In addition to the lack of intramolecular rotational problems, cyclohexyl trifluoroacetate does not have the rigid structure of the previous two molecules. A flexing of the cyclohexane ring would be sufficient to cause resolution problems. The latter may never arise, however, as the liquid crystal should

serve to hold the ring in a rigid chair form.

The dipolar-coupled spectrum of cyclohexyltrifluoroacetate contains 12 well-resolved lines. This indicates that the solution was computationally extractable although tedious. The results of these multiple calculations are in Figure 7. It can be seen in this figure that the acetate moiety is in the antipicated, or what may be termed a chemically sensible, location. The rotation about the C1–O bond was well behaved as the *R* factor dropped to a minimum of 40°. A 5° rotation away from this minimum produced a rejectable model at the 99.99% confidence interval. This high precision would indicate that the cyclohexane ring is rather tightly held within the liquid crystal. In contrast to this well-behaved feature, the second rotational mode (around the O—C=O bond) was poorly behaved. The best location was found to be at 50° and could not be fixed closer than 10°. This is the same type of behavior that was found in the acetyl case. Again, we can only rationalize these results on the basis of the small steric size of the trifluoromethyl moiety. As might be expected, the smaller carbon skeleton of the cyclohexane relative to the size of the [2,2,1] allows the liquid crystal to approach more closely the trifluoromethyl moiety. This is indicated by the 10° rotational freedom for the cyclohexyl trifluoroacetate vs. the 20° rotational freedom for acetyl. As was the case before, the averaged coordinates of all the rotational models proved to be unsatisfactory at the 99.95% confidence interval.

Conclusions

An attempt was made to show that the ^{13}C to ^{19}F dipolar coupling experiment is a usable structure determination technique. The ability to use the trifluoromethyl moiety as the source of ^{19}F greatly expands the utility of this technique. It may appear that the application of the technique is extremely tedious but this may be avoided. In the *p*-difluorobenzene experiment, the structural refinement could have been done in less than 2 working days—one to obtain the spectra and the second to preform the calculations. The amount of time required could be even shorter with a wide-bore superconducting NMR. The calculations for the other two examples took considerably longer than 1 working day, due to exploration of the rotational behavior of the functionalities as they were affected by the nematic solvent. This tedious exploration could have been eliminated if potential abnormal behavior had not been investigated. Future users need not be so suspicious.

From these results, it is obvious that this structure determination method cannot be used to determine the rotational populations of a mobile substrate, as the liquid crystal restricts the substrate in what may be an abnormal orientation. This restriction plus the need for a source of ^{19}F are the two major limitations to this technique. On the other hand, the technique produces reproducible, statistically and chemically defendable structures. The technique should find wide applications in the biomedical studies of lipid-bound systems.

Acknowledgment. This work was supported by the National Institutes of Health under the MBRS program, Grant 2 S06 RR08047-13.

Registry No. *p*-Difluorobenzene, 540-36-3; 3-(trifluoroacetyl)camphor, 51800-98-7; cyclohexyl trifluoroacetate, 1549-45-7.

α-Amino Acids as Chiral Educts for Asymmetric Products. Chirospecific Syntheses of the 5-Butyl-2-heptylpyrrolidines from Glutamic Acid

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Both enantiomers of *trans*-5-butyl-2-heptylpyrrolidine, an active and major component in the repellent venom of the ant *Solenopsis fugax*, have been synthesized with very high diastereomeric and optical purity from glutamic acid. Both enantiomers of the cis isomer also have been synthesized in an extension of our methodology to encompass the preparation of both cis and trans, optically pure, 2,5-disubstituted pyrrolidines and because of their potential entomological interest. Initially, a sulfide contraction process efficiently introduces the first side chain onto a pyroglutamate intermediate. Various strategies to elaborate the second side chain have been developed along with methods to control and establish the relative stereochemistry at C-2 and C-5 of the pyrrolidine ring with high selectivity. 2,5-Dialkyl-1-pyrrolines, which also have been identified in the ant venom, can be prepared by these processes as well with specific absolute stereochemistry.

Various unsymmetrical *trans*-2,5-dialkylpyrrolidines have been identified as the major components in the venom released from a variety of ants in the genus *Solenopsis*.[1] These ants secrete their venom as a powerful repellent to ward off defending host ant species whose nests these thief ants raid for the larvae. Due to this entomological interest, various methods to prepare *trans*-2,5-dialkylpyrrolidines have appeared in the literature. However, these earlier syntheses[2] did not achieve any degree of selectivity in establishing the relative stereochemistry about the pyrrolidine ring. The final mixtures obtained from these routes contained nearly equal amounts

(1) Jones, T. H.; Blum, M. S.; Fales, H. M. *Tetrahedron* **1982**, *38*, 1949 and references cited therein.

(2) (a) Frazer, R. R.; Passannanti, S. *Synthesis* **1976**, 540. (b) Pedder, D. J.; Fales, H. M.; Jaouni, T.; Blum, M.; MacConnell, J.; Crewe, R. M. *Tetrahedron* **1976**, *32*, 2275. (c) Jones, T. H.; Blum, M. S.; Fales, H. M. *Tetrahedron Lett.* **1979**, 1031. (d) Jones, T. H.; Franko, J. B.; Blum, M. S.; Fales, H. M.; *Tetrahedron Lett.* **1980**, *21*, 789. (e) Schmitz, E.; Sonnenschein, H.; Grundemann, C. *J. Prakt. Chem.* **1980**, 322, 261. (f) Blum, M. S.; Jones, T. H.; Holldobler, B.; Fales, H. M.; Jaouni, T. *Z. Naturwissensch.* **1980**, *67*, 144.

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