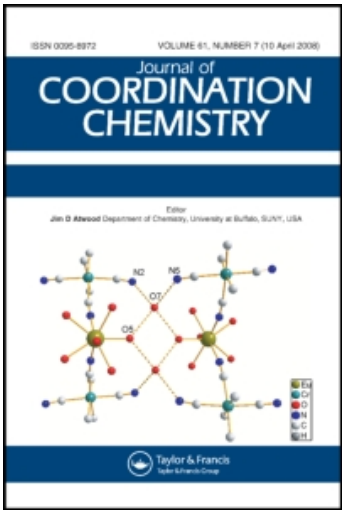

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D. André D'avignon[a]; Cathleen E. Hanau[a]; Yvette M. Fobian[a]; Kevin D. Moeller[a]
[a] Department of Chemistry, Washington University, St. Louis, MO

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APPLICATION OF HMBC AND HMQC-TOCSY NMR METHODS TO ASSIGN THE STRUCTURES OF BICYCLIC-PEPTIDE MIMETICS

D. ANDRÉ D'AVIGNON*, CATHLEEN E. HANAU, YVETTE M. FOBIAN and KEVIN D. MOELLER

Department of Chemistry, Washington University, Campus Box 1134 St. Louis, MO 63130

The structures of representative bicyclic peptides are confirmed through the NMR methods of HMBC and HMQC-TOCSY. Complete assignment of proton and carbon resonances is afforded by these two-dimensional NMR methods. HMQC-TOCSY is especially useful for assigning spectra in molecules having extensive proton spin systems and in establishing connectivities between protonated carbons. Long-range proton-carbon connectivities obtained by HMBC confirm structure in molecules containing heteroatoms or non-protonated carbons that interrupt proton spin systems.

KEYWORDS: NMR, bicyclic peptides, HMBC, HMQC-TOCSY

INTRODUCTION

In recent years, a number of powerful NMR methods (HMQC, HMBC, TOCSY, and HMQC-TOCSY, to mention a few) have been used to greatly simplify the assignment of complex spectra.[1] These methods, when combined with limited chemical information, provide, in many cases, a means to assign molecular structures in a relatively straightforward and systematic fashion. Advances in NMR hardware such as reverse-detection and micro-probe technology have considerably reduced spectrometer time and sample requirements such that it is now practical to routinely implement these informative and time-saving methods. In this contribution we present selected applications of HMBC and HMQC-TOCSY to assign structures of conformationally restricted bicyclic-peptide building blocks. Commonly, the bicyclic peptide products are mixtures of stereoisomers that yield complex one-dimensional NMR spectra. Multidimensional NMR methods represent the only viable route to probe the solution structure of these chemical systems. It is important to mention that in the past three to four years, numerous reverse-detected NMR strategies requiring isotopic enrichment for ^{13}C and ^{15}N have been proposed.[2] These strategies have been very successful in characterization of biopolymers. However, all of the work presented here was carried out on unlabeled samples containing NMR active nuclides (^{1}H,^{13}C) present at natural abundance.

The NMR methods used in this work are two-dimensional schemes that take advantage of through-bond polarization transfer from a highly receptive ^{1}H nucleus to the less abundant ^{13}C nucleus.[3] The proton-detected multiple-quantum coherence (HMQC) method provides a map of one-bond proton-carbon connectivities.[4]

Proton-detected multiple-bond coherence (HMBC) provides long-range correlations, primarily two- and three-bond connectivities between protons and carbon.[4] Occasionally, four- or five-bond connectivities can be observed by HMBC where long-range coupling constants are appreciable or relaxation rates are favorable. HMBC can be the method of choice to assign structure in molecules containing heteroatoms or non-protonated carbons that interrupt extended proton spin systems. The HMQC-TOCSY[5] experiment yields HMQC information plus addition TOCSY[6] generated connectivities originating from proton-proton scalar couplings. These additional connectivities encode two- (or greater) bond proton-carbon interactions and thus, indirectly, yield the highly informative protonated carbon-protonated carbon bond assignments. In molecules where many carbon atoms are bound to hydrogen, the HMQC-TOCSY method can, in principal, be used to generate a map of carbon-carbon bonding at concentrations as low as 1 mM. This information can be used to generate a carbon skeleton of a molecule of interest. HMQC-TOCSY can be a method of choice when the proton spectrum is congested and dispersion in the carbon chemical shift spectrum is frequently much improved. In a simplistic view, the HMQC-TOCSY experiment can be viewed as taking the proton-proton coupling information buried in a complex proton NMR spectrum and spreading it over the ^{13}C chemical shifts. Steroids, alkaloids, and molecules such as those reported here, are ideal candidates for HMQC-TOCSY spectroscopy.

EXPERIMENTAL

All NMR spectra were recorded using a VXR-500 (Varian Associates, Palo Alto, CA) multinuclear NMR spectrometer equipped with a model nmr-5M3L radio-frequency amplifier (ENI, Rochester, NY) and an indirect detection probe (Nalorac Corp., Martinez, CA). Typical 90° flip angle pulses were 8.0 msec (^{1}H) and 19 msec (^{13}C). NMR spectra were recorded at 25°C, unless noted otherwise, on 20–40 mg of sample dissolved in 0.5 mL deuterochloroform (Cambridge Isotopes, Woburn, MA).

HMQC Spectroscopy

One-bond ^{1}H-^{13}C shift correlation spectroscopy was recorded using the pulse sequence proposed by Bax and Subramanian.[7] The spectrum was acquired as $2048 \times 200 \times 2$ points with spectral windows of 2548.3 Hz in t_2 (^{1}H axis) and 10354.6 Hz in t_1 (^{13}C shift axis). A delay time of 1.5 s was used between each of four scans per t_1 increment. ^{13}C Waltz decoupling was employed during acquisition with a power sufficient to decouple over 12,000 Hz. Data were zero-filled to 2048×1024 and processed by the method of States, *et al.*[8] with Gaussian apodization in both dimensions prior to Fourier transformation.

HMBC Spectroscopy

The multiple bond ^{1}H-^{13}C shift correlation spectra were recorded under conditions similar to HMQC except 16–32 transients are collected, a delay of 55 ms was used for multiple-quantum coherence to develop, and magnitude mode sine-bell apodization was used in the t_2 dimension prior to Fourier transformation.

HMQC-TOCSY Spectroscopy

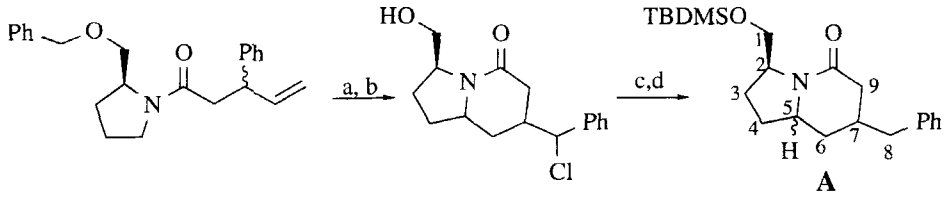
The single-bond ^{1}H-^{13}C shift correlation-relay experiment was carried out by employing the method of Lerner and Bax[6] with parameters similar to HMQC with the following exceptions: The TOCSY spin lock period was carried out using MLEV-17 for 8–22 ms duration using a field strength of 11 kHz. Commonly 16 transients were collected and data processing followed identical to that employed for HMQC.

Organic Synthesis

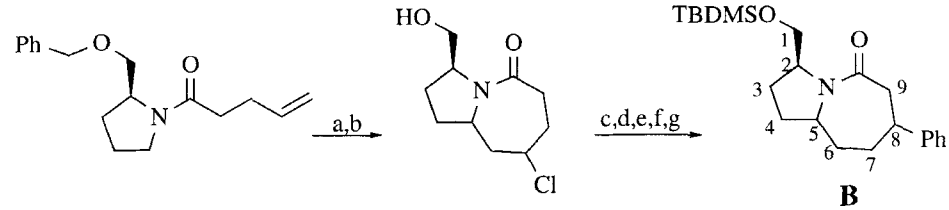
The synthetic details of all molecules include in Scheme 1, 2, and 3 are unpublished and will be included in forthcoming manuscripts.

RESULTS AND DISCUSSION

Schemes 1–3 represent a series of synthetic routes to conformationally restricted bicyclic peptide building blocks that capitalize on oxidative electrochemistry. In each of the routes, a simple amino acid derivative was selectively functionalized at an anode surface, and then the product cyclized to form the bicyclic ring skeleton. In Scheme 1, the cyclization step was expected to lead to a seven-membered ring



a) C rod anode, Pt wire cathode, 0.03 M Et_4NOTs, 26.3 mA, 2.5 F/mole, 60% yield. b) titanium tetrachloride (1M in CH_2Cl_2), CH_2Cl_2, −78°C to rt. 42 hr, 81% yield. c) 5% Pd/C, NaOMe, MeOH, H_2, 24 hr, 87% yield. d) TBDMSCl, imidazole, DMF, 24 hr, 81% yield.



a) C rod anode, Pt wire cathode, 0.03M Et_4NOTs, MeOH, 42mA, 2.25F/mole, 82% yield. b) titanium tatrachloride (1M in CH_2Cl_2), CH_2Cl_2, 82% yield. c) Ra-Ni, H_2, KOH, MeOH, 84% yield. d) TBDMSCI, DBU, CH_2Cl_2, 91% yield, diastereomers seperated 1:1. e) i) LDA, ii) PhSSPh, THF, 82% yield. f) i) MCPBA, ether, ii) 90°, toluene, $CaCO_3$, 62% yield. f) $Ph_2CuCNLi_2$, $BF_3 \cdot Et_2O$, Et_2O, 42% yield.

lactam. However, HMQC and HMQC-TOCSY experiments demonstrated that an unexpected rearrangement reaction occurred. The final product (A) clearly possessed a six-membered ring lactam. By performing HMQC-TOCSY experiments on each product in this reaction sequence, it was determined that the rearrangement had occurred in the Lewis acid catalyzed cyclization step (Scheme 1, step b). Since product A was a mixture of diastereomers, the one-dimensional ^1H NMR was complicated (Fig. 1) making the identification of the 5,6 lactam extremely difficult in the absence of HMQC-TOCSY data.

Figure 1 shows the HMQC plot revealing cross peaks correlating one-bond proton-carbon interactions for product A. Product A was a mixture of diastereomers in an approximately 60/40 yield. A number of methylene group resonances were assigned immediately by finding two cross peaks (contours) corresponding to a single carbon resonance. The HMQC-TOCSY plot of A (Fig. 2) shows HMQC cross peaks (one-bond H,C connectivities) as well as additional features stemming from isotropic mixing of proton magnetization to nearby protons. In this particular example, the TOCSY mixing period was chosen to be 15 ms thus facilitating significant mixing only between protons that exhibit strong coupling (two or three bonds removed). Because all coupled protons in A were attached to carbons, the HMQC-TOCSY experiment also indirectly yielded the carbon-carbon framework of the product molecule and thus established either the 5,6- or 5,7-bicyclic product. It

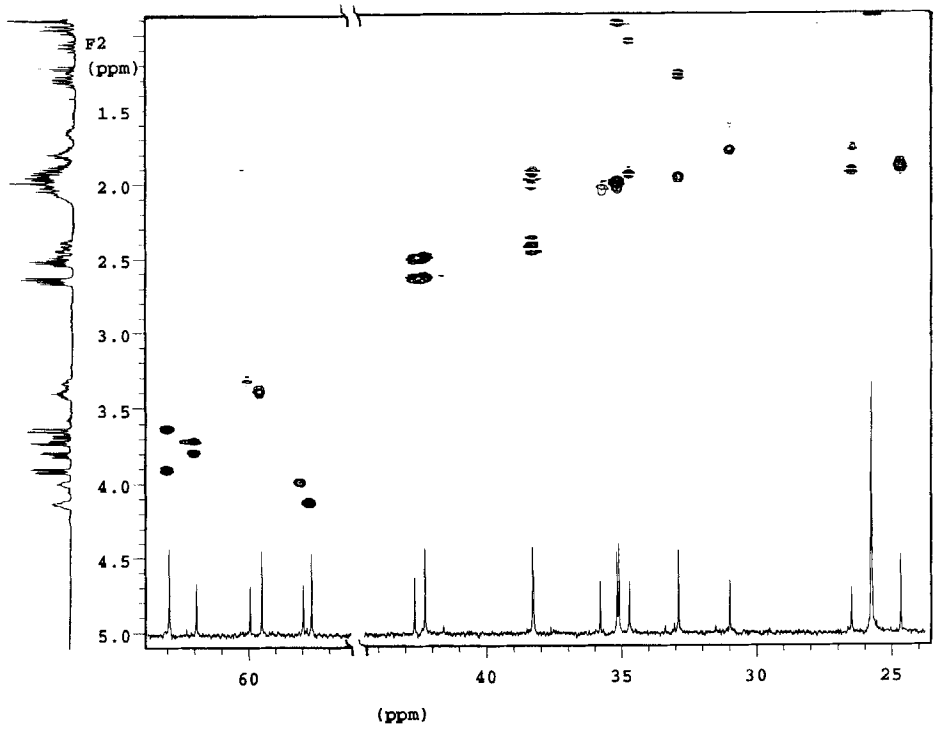


Figure 1 HMQC spectrum of product A in $CDCl_3$ recorded at 500 MHz at 25°C. One dimensional ^1H and ^{13}C spectra are shown along the F1 and F2 axes, respectively.

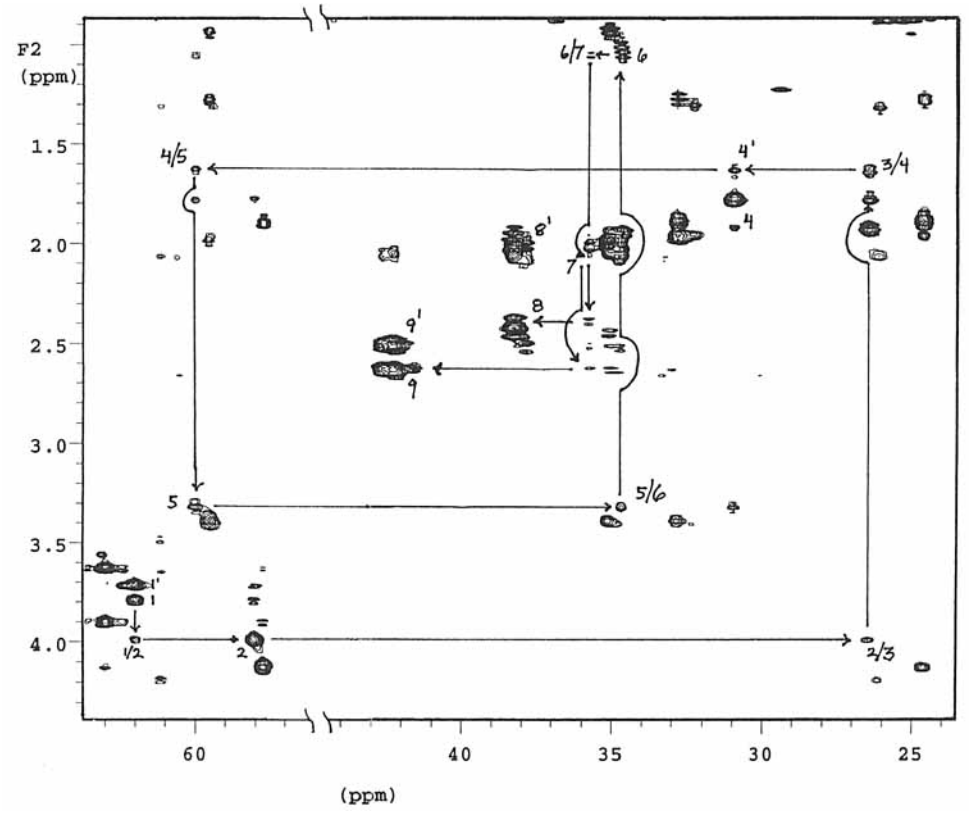


Figure 2 HMQC-TOCSY spectrum of product A in $CDCl_3$ recorded at 500 MHz at 25°C. The arrows indicate the assignment pathway for the minor isomer. The numbers correspond to resonance assignments from Scheme 1. In cases where non-equivalent protons are bound to a single carbon, the cross peak is labeled with both a primed and non-primed number. The relayed peaks are labeled by both atom positions. For example, the contour indicating relayed coherence between protons on atoms 2 and 3 is labeled 2/3.

is this feature that makes the HMQC-TOCSY method especially useful in characterizing bicyclic molecules. The assignment for the minor isomer was made in the following way. The methylene resonance at approximately 62 ppm was assigned to carbon #1 of the minor isomer and was chosen as a convenient starting point (see numbering Scheme 1 and Figure 2). The additional cross peaks found for HMQC-TOCSY, compared to HMQC, were used to "walk" around the molecule and assign resonances to successively bound carbon and proton atoms. The #2 position carbon and attached protons were assigned by locating the additional cross peaks along either the vertical (F_2) axis, or the horizontal (F_1) axis of the #1 position cross peak (see arrow indicating the relayed connectivity labeled 1/2 at 62 ppm). Carbon #3 was located in a similar fashion (26.5 ppm) by looking for the cross peak appearing along the F_1 axis at the proton chemical shift of H2. This approach was used to assign all the connectivities for the minor isomer. Major isomer assignments were made as well but are complicated somewhat by the near degeneracy of carbons

6 and 7. The HMQC-TOCSY spectrum was consistent only with the 5,6-bicyclic product as drawn in Scheme 1. An independent synthesis of the 5,7-bicyclic product B (single isomer) was carried out (Scheme 2) and the resulting HMQC and HMQC-TOCSY plots and labeled assignments are shown in Figures 3 and 4. Product B showed carbon 7 coupled to only two carbons (6 and 8), unlike Product A, which showed carbon 7 connected to carbons 6, 8, and 9. The data shown in Figures 3 and 4 are somewhat cleaner than that of Figures 1 and 2 due to the presence of only one stereoisomer and excellent carbon shift dispersion. It is significant that HMQC-TOCSY is the only method which gave us unambiguous information allowing differentiation between the 5,6- and the 5,7-bicyclic products. In the absence of HMQC-TOCSY data, the product would have been assigned to the 5,7 bicycle structure in analogy to earlier experiments.[9]

The HMQC-TOCSY method is especially well-suited to assign spectra from molecules with extensive proton spin systems. All of the intermediate products in Scheme 1 fall into this category and have been characterized by HMQC-TOCSY. In one intermediate, for example, four isomers (two sets of diastereomers) were present and assignments were successfully made for all proton and carbon resonances in what was a very complex one-dimensional ^1H spectrum. Ultimately, the real power

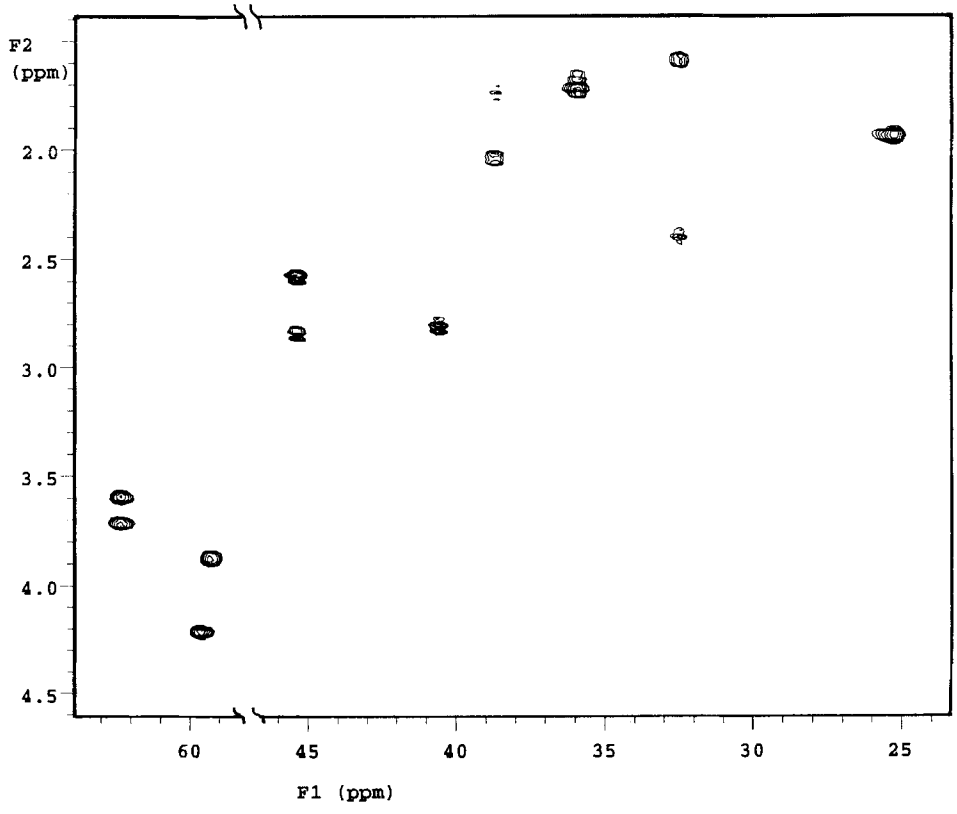


Figure 3 HMQC spectrum of product B in $CDCl_3$ recorded at 500 MHz at 25°C.

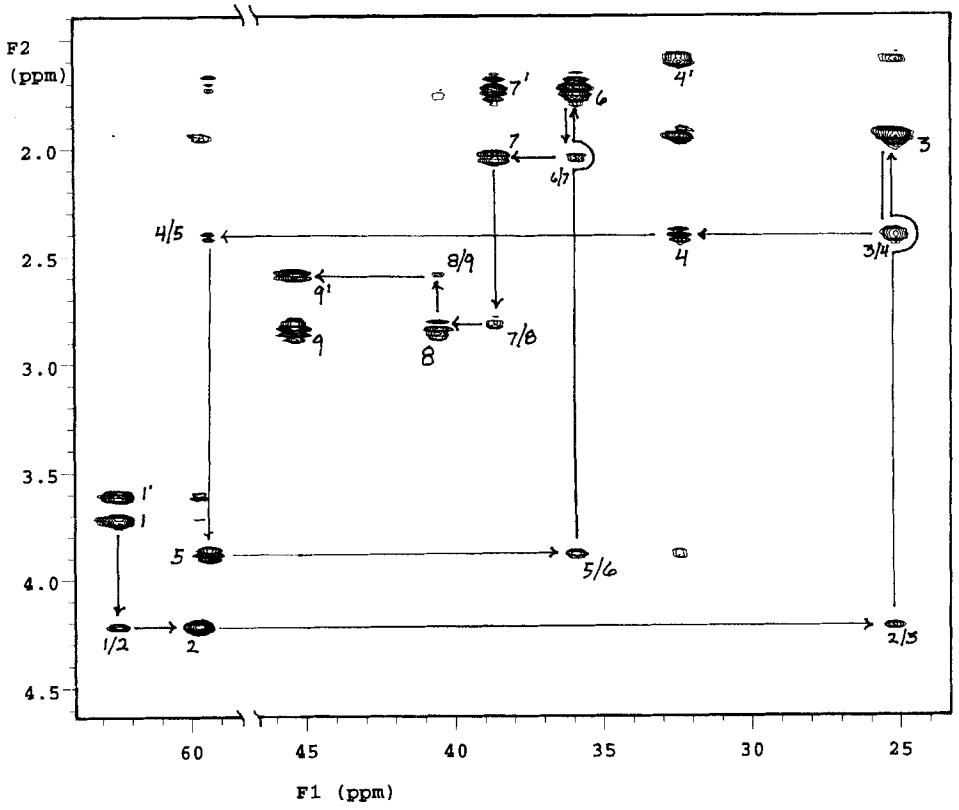
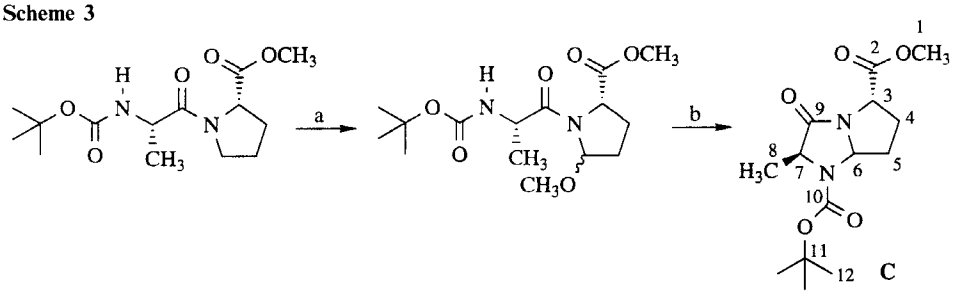


Figure 4 HMQC-TOCSY spectrum of product B in $CDCl_3$ recorded at 500 MHz at 25°C. The arrows indicate the assignment pathway and numbers are correlated to the atom position assignments in Scheme 2.

of HMQC-TOCSY is in carbon-carbon bond information that can be obtained on milligram quantities of many small to intermediate-sized molecules. This is in sharp contrast to the INADEQUATE[10] method which requires at least 200 mg of sample to obtain carbon-carbon connectivity information in a reasonable time period. The INADEQUATE method takes advantage of a direct carbon-carbon multiple quantum coherence that is very small due to the low natural abundance of carbon. Carbon assignments *via* HMQC-TOCSY are limited to protonated carbon-protonated carbon connectivities, as the carbon coherence encoded in its attached proton is relayed to an adjacent coupled proton. However, combining HMBC with HMQC-TOCSY can allow one to assign non-protonated carbons.

In Scheme 3, an electrochemical oxidation-based route to a 5,5 bicyclic peptide mimetic (C) is shown. From an NMR perspective, product C differed from A and B in that the extensive proton-proton spin system was interrupted by nitrogen. HMQC-TOCSY could be used to assign the 3-6 positions but, because of the limitations described above would not in itself confirm the bicyclic ring closure. In this case, an HMBC experiment was used to assign the carbons and hydrogens as

Scheme 3



a) anodic oxidation, MeOH, Et_4NOTs, sharpened C rod anode/Pt wire cathode, 56.2 mA, 17.5 F/mol, 76%. b) BF_3-Et_2O, Et_2O, 0°C, 15 min, 54%.

well as to confirm the ring closure. The HMBC connectivities are given in Table 1, and the observed long-range correlations are indicated in Figure 4. For product C, the HMBC experiment was carried out at −4°C where rotomers about the N-CO_2-linkage are frozen out. At 25°C, the rotomer exchange was sufficiently rapid to short-circuit the multiple quantum coherence between protons 6 and 7 to carbon 10.

CONCLUSION

In summary, HMQC, HMQC-TOCSY, and HMBC experiments have proven to be powerful tools for elucidating the structures of several conformationally restricted bicyclic peptide building blocks. These techniques derive their utility from their ability to dramatically simplify the assignment of molecules with extremely complex proton nmr spectra. It is clear that two-dimensional NMR data of this type will play a pivotal role in the future assignment of all new structures in this growing family of peptide mimetics.

Table 1 HMBC Long-range correlations observed for product C

position (scheme)	^1H chemical shift (ppm)	carbon atom positions (ppm) to which long-range correlations were observed
1	3.68	2 (171.6)
3	4.46	2 (171.6), 4 (29.1), 6 (75.9), 9 (173.4)
4	2.05	2 (171.6), 3 (54.4), 5 (33.1), 2 (171.6),
	2.48	3 (54.4), 5 (32.8), 6 (75.9)
5	1.34[a]	——
	2.34	3 (54.4), 4 (29.1), 6 (75.9)
6	5.18	5 (32.8), 8 (17.9), 9 (173.2), 10 (152.6)
7	4.25	5 (32.8), 6 (75.9), 8 (17.9), 9 (174.2), 10 (153.3)
8	1.34	7 (57.4)
12	1.40	11 (80.9)

[a] Correlations from the proton at position 5 (1.34 ppm) were absent or obscured by the more intense signal at position 8 (1.34 ppm).

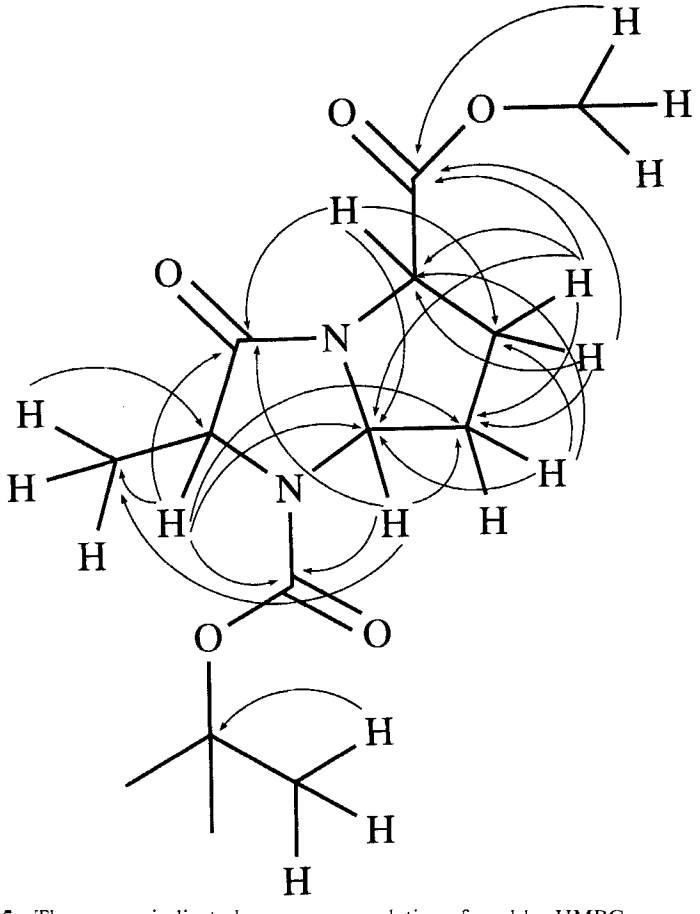


Figure 5 The arrows indicate long-range correlations found by HMBC on product C.

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