First Synthesis of a Fully [^{15}N,^{13}C]Backbone-Labelled Peptide. ^{15}N NMR Spectrum of Corresponding Leu-Enkephalin

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Leu-Enkephalin, fully labelled with ^{13}C and ^{15}N nuclei in the backbone, is prepared chemically and the corresponding heteronuclear scalar coupling parameters measured from its ^{13}C and ^{15}N NMR spectra.

As a first application of our amino acids[1,2] labelled with stable isotopes (^{15}N,^{13}C), we here report a synthesis of fully [^{15}N,^{13}C]backbone-labelled Leu-enkephalin **5**. Moreover, we have undertaken a preliminary examination of one-dimensional ^{13}C and ^{15}N NMR spectra of this new enkephalin isotopomer. Our major aim was to explore the scope of this type of isotope labelling for the determination of heteronuclear coupling constants required in structural investigations of peptides.[3]

The peptides were prepared by a stepwise approach in solution from the [^{15}N, 1, 2-$^{13}C_2$]labelled Boc-amino acids[2] using TBTU as the condensing agent.[4] The coupling steps were in all cases complete (TLC) within 1 to 2 h, thus furnishing the pure protected intermediates **1–4** in 86–94% yields. After subsequent deprotection of **4** by hydrogenolysis and acidolysis (HCl), simple reprecipitations yielded the desired free Leu-enkephalin **5** in pure form as its hydrochloride salt, which was used as such in NMR experiments.

The starting point for our NMR analysis of **5** was the assignment of the five non-overlapping ^{15}N resonances, four of which fall within a 13 ppm range, to specific amino acid residues. For this purpose, we have compared our data with those previously reported by Roques *et al.*,[5] who assigned the nitrogens of the two glycines, phenylalanine and leucine of a ^{15}N tetralabelled Leu-enkephalin isotopomer. For **1–4**, we have observed the resonances of Leu_5, Phe_4, Gly_3 and Gly_2 at 117.6–118.7, 115.4–116.9, 103.4–105.9 and 105.9 (Table 1), respectively. In the light of the above data, which are in agreement with previous correlations of ^{15}N peptide shifts,[6,7] we have assigned the nitrogen resonances of **5** as indicated in Table 1.

Inspection of the ^{15}N signals of **5** reveals that the doublet at δ 40.5 (J 6.2 Hz), assigned to the ^{15}N nucleus of the *N*-terminal tyrosine residue, is due to coupling to the corresponding C_α atom. Further examination of the amide ^{15}N systems (Fig. 1), as exemplified by Gly_2, shows that the ^{15}N atom interacts with the ^{13}CO ($^1J_{CON}$ 15.7 Hz) and the $^{13}C_\alpha$ ($^2J_{C\alpha N}$ 9.8 Hz) of the preceding residue. The third coupling arises from the intra-residue correlation between the ^{15}N atom and the $^{13}C_\alpha$ (^1J 11.1 Hz). This is valid for any ^{15}N nucleus involved in a peptide bond and illustrates that the scalar couplings obtained in this simple experiment are useful as evidence for the direct attachment of two amino acids in a given dipeptide unit of **5**. Such correlations are otherwise generally achieved by application of NOE experiments or multidimensional NMR methods.[8–11]

As shown in Fig. 1, the above heteronuclear coupling constants can also be deduced readily from the corresponding ^{13}C spectrum. With the exception of the *C*-terminal Leu_5, the carbonyl ^{13}C signal of which appears as a doublet at δ 173.7 ($^1J_{C\alpha CO}$ 58.7 Hz), the ^{13}CO groups appear as double doublets (Fig. 1). This pattern arises from the fact that each peptide bond carbonyl is coupled to the corresponding $^{13}C_\alpha$ ($^1J_{COC\alpha}$ 50–53 Hz) as well as to the ^{15}N atom of the following residue in the peptide sequence ($^1J_{CON}$ 14–16 Hz). Likewise, the scalar couplings extracted from the C_α systems of **5** give the connectivity between the C_α of any residue i and the ^{15}N atom of the corresponding $i + 1$ fragment ($^2J_{C\alpha N}$ 8–11 Hz). This allows the discrimination between ^1J (11–11.5 Hz) and ^2J (8–11 Hz) obtained from the ^{15}N spectrum and therefore facilitates the identification of a dipeptide fragment in cases

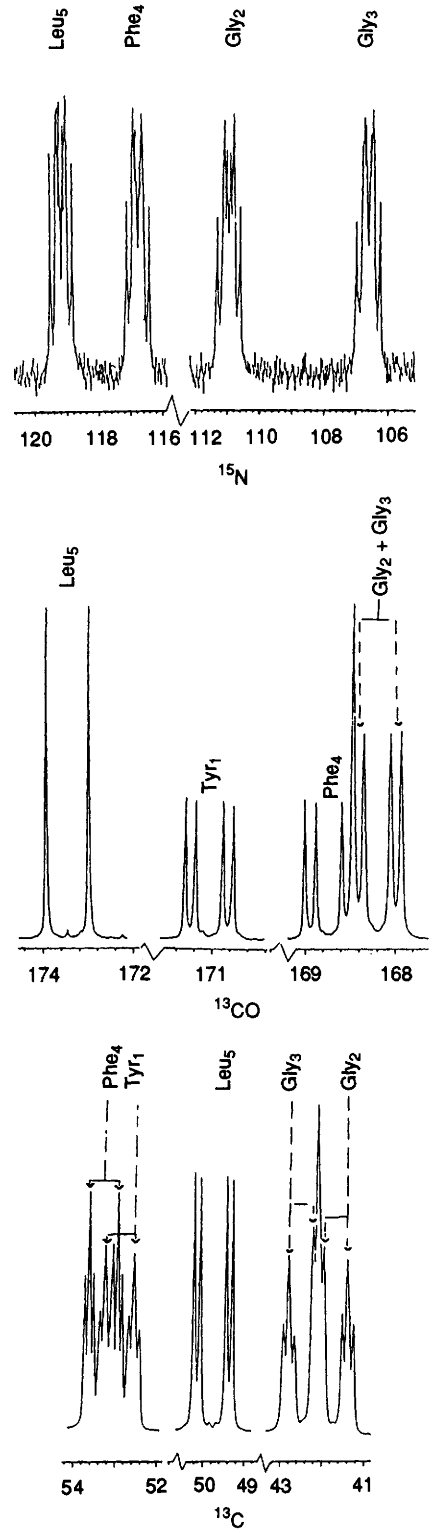

Fig. 1 Relevant parts of the ^{15}N (at 50.66 MHz) and ^{13}C (at 124.98 MHz) spectra of fully [^{15}N,1,2-$^{13}C_2$]backbone-labelled Leu-enkephalin **5**

Table 1 ^{13}C (CO and C_α regions) and ^{15}N NMR data of compounds **1–5**

		1 [δ, multiplicity, *J*/Hz]	**2**	**3**	**4**	**5**
Leu	CO	172.3, d, 61.3	172.1, d, 61.3	172.4, d, 61.0	172.1, d, 62.3	173.7, d, 58.7
	C_α	50.8, dd, 11.6; 61.6	51.0, dd, 11.6; 61.0	50.9, dd, 11.6; 61.4	50.5, dd, 11.6; 61.3	50.0, dd, 10.9; 58.7
	N	117.6, ddd, 8.6; 11.7; 15.0	118.7, ddd, 8.4; 11.6; 15.0	118.7, ddd, 8.9; 11.4; 14.5	118.3, ddd, 9.3; 11.1; 14.1	119.1, ddd, 8.9; 11.2; 14.5
Phe	CO	170.9, dd, 52.5; 15.0	170.4, dd, 15.3; 53.1	171.1, dd, 14.9; 53.1	171.4, dd, 14.7; 53.4	168.7, dd, 16.0; 52.8
	C_α	55.6, bd, 52.5	54.0, dt, 10.0; 53.1	54.0, dt, 10.4; 53.1	53.5, dt, 10.4; 53.1	53.8, dt, 8.4; 52.8
	N	88.6, dd, 0.6; 11.7	115.4, ddd, 8.7; 11.6; 15.5	116.9, ddd, 9.5; 11.4; 15.0	116.8, ddd, 10.1; 11.3; 14.2	116.7, ddd, 9.7; 11.1; 14.6
	OCO	155.4, dd, 3.0; 25.6				
Gly_3	CO		169.3, dd, 15.3; 53.1	169.6, dd, 15.9; 52.5	169.1, dd, 15.2; 52.5	168.2, dd, 15.0; 52.0
	C_α		44.2, dt, 10.0; 53.1	43.7, dt, 11.0; 53.4	42.0, dt, 10.4; 53.1	42.0, dt, 9.7; 50.4
	N		76.1, bd, 12.8	103.4, ddd, 9.3; 12.6; 15.3	105.9, unresolved m	106.6, ddd, 10.1; 11.5; 15.0
	OCO		156.0, bd, 26.8			
Gly_2	CO			168.4, dd, 15.9; 52.5	168.2, dd, 14.7; 52.5	168.2, dd, 15.0; 52.0
	C_α			42.9, dt, 11.0; 53.4	41.6, dt, 11.0; 51.9	41.6, dt, 9.7; 50.4
	N			78.2, d, 13.3	105.9, unresolved m	110.9, ddd, 9.8; 11.1; 15.7
	OCO			156.1, d, 28.0		
Tyr	CO				172.1, dd, 14.7; 53.4	171.0, dd, 14.2; 52.0
	C_α				55.9, dt, 11.0; 53.7	53.6, dt, 9.7; 52.4
	N				90.4, d, 11.6	40.5, d, 6.2
	OCO				155.3, d, 24.4	

All amino acids are [^{15}N, 1,2-$^{13}C_2$] labelled; **1**, Boc-Phe-Leu-OBzl; **2**, Boc-Gly-Phe-Leu-OBzl; **3**, Boc-Gly-Gly-Phe-Leu-OBzl; **4**, Boc-Tyr(Bzl)-Gly-Gly-Phe-Leu-OBzl; **5**,HCl·Tyr-Gly-Gly-Phe-Leu. Spectra recorded [$SiMe_4$ (ref. ^{13}C) internal reference and $HCO^{15}NH_2$ (δ 113.2 ^{15}N) external reference]: ^{13}C at 67.5 MHz and ^{15}N at 9.03 MHz in $CDCl_3$ for **1–3** (full resolution in ^{15}N spectrum of **3** at 50.66 MHz in $CDCl_3$); ^{13}C at 124.98 MHz and ^{15}N at 50.66 MHz in $(CD_3)_2SO$ for **4** and free pentapeptide **5**.

of overlapping.[9]

As is evident from Table 1 and Fig. 1, the ^1H-irradiated ^{15}N and ^{13}C spectra of **5** exhibit a multitude of specific couplings originating from spin–spin interactions of its ^{15}N and ^{13}C labels. Several such homo- and hetero-nuclear couplings contain valuable information useful in conformational analysis of peptides.[3] As the spectral properties of fully enriched, backbone-labelled oligopeptides have not hitherto appeared in the literature, the depicted ^{13}C and ^{15}N NMR spectra serve as a conspicuous illustration of their particular features. Furthermore, this preliminary NMR study amply demonstrates the scope of isotope-labelled peptides in miscellaneous structural investigations.

Financial support from the Swedish Natural Science Research Council, the National Board for Industrial and Technical Development and the Carl Trygger Foundation as well as a scholarship (to B. N.) from the International Science Programs are gratefully acknowledged. We thank Charlotta Damberg at the Swedish NMR Center for the spectra.

Received, 23rd June 1994; Com. 4/03812F

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