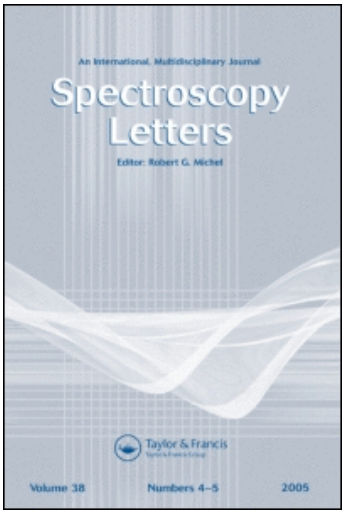

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^{1}H and ^{13C} NMR Determination of the Enantiomeric Purity of Substituted 4-Amino-3- (thien-2-Yl)-Butyric Acids and 4-Amino-3-(Benzo[b]Furan-2-yl)-Butyric Acids, Ligands of the $Gaba_B$ Receptor.

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^{1}H AND ^{13}C NMR DETERMINATION OF THE ENANTIOMERIC PURITY OF SUBSTITUTED 4-AMINO-3-(THIEN-2-YL)-BUTYRIC ACIDS AND 4-AMINO-3-(BENZO[B]FURAN-2-YL)-BUTYRIC ACIDS, LIGANDS OF THE $GABA_B$ RECEPTOR.

KEY-WORDS

4-Amino-3-(benzo[b]furan-2-yl)- Butanoic Acids ; 4-Amino-3-(thien-2-yl)- Butanoic Acids; ^{1}H and ^{13}C NMR; 2D NMR; enantiomeric composition; chiral derivatization; (*S*)-(-)-α-methylbenzylamine

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ABSTRACT

The enantiomeric composition and absolute configuration of 4-Amino-3-(benzo[b]furan-2-yl)- Butanoic Acids and of 4-Amino-3-(thien-2-yl)- Butanoic Acids **1** may be accurately determined by ^{1}H and ^{13}C nuclear magnetic resonance analysis of the corresponding derivatives **3** prepared by reaction with chiral reagents. Correlation with HPLC is signaled.

INTRODUCTION

The neutral amino acid, γ-aminobutyric acid (GABA) is an inhibitory neurotransmitter concerned with the control of neuronal activity in the mammalian central nervous system and with the regulation of many physiological mechanisms [1]. Within the central and peripheral nervous systems, GABA has been shown to act through at least two distinctly different receptor sites [2]. These are termed $GABA_A$ and $GABA_B$ receptors, with different binding properties [3,4]. Until now, β-p-chlorophenyl-GABA (baclofen) is one of the reference selective agonist for the $GABA_B$ receptor [5]. The enantiomers of baclofen were found to have quite different properties. The efficiency in spasticity has been attributed to the (-)*R* baclofen [3,6]. The isolation of the pure biologically active constituent responsible for the properties is essential for studying the mode of action [7].

In previous papers [10,20,22, 24] we described the synthesis of 3-(benzo[b]furan-2-yl)-GABA **1a-c** and 3-(thien-2-yl)-GABA **1d-f**, new discriminated ligands of $GABA_B$ sites, which are specific $GABA_B$ receptor antagonists [19,21,23] or agonists [25,26] and some are now commercially available as racemates (from Tocris Cookson, England).

Enantiomeric purity is often quantified through HPLC [8]. This paper describes the results of ^1H and ^{13}C NMR analysis of **1** after derivatization. Each component was characterized by ^1H and ^{13}C NMR, anisochronous resonances being used as probes for quantitation and absolute structural assignment. This study is correlated by HPLC resolution.

EXPERIMENTAL

IR spectra were recorded in the solid state (KBr pellet) on a Beckman-Acculab 4 spectrometer, wavenumbers are expressed in cm^{-1}. Routine ^1H (80.13 MHz) NMR spectra were run on a Bruker WP 80 spectrometer. The structure of all the diastereomers **3** was established by ^1H and ^{13}C NMR spectroscopy in $CDCl_3$ (*ca* 0.05 M) on a Bruker AC 300 spectrometer operating respectively at 300.133 MHz and 75.469 MHz, using a 5 mm dual $^1H/^{13}C$ probehead at 25 °C (Laboratoire d'Application RMN - Université Lille II). Chemical shifts are expressed downfield from TMS (0 ppm). The complete assignments of ^1H and ^{13}C NMR signals were performed by proton decoupled DEPT and 2D NMR experiments. Proton decoupled DEPT spectra [12] (32 K) were acquired using decoupler channel proton pulses of 135° (13.5 μs) with a delay between pulses of 1s. Exponential multiplication prior to Fourier transform was performed with line broadening values of 5 Hz. The homonuclear chemical shift correlation 2D experiments [13] (program COSY AUR) were carried out by using the pulse sequence: t_2 - 90°[^1H] - t_1 - 45°[^1H] - FID[^1H]. Relaxation delay t_2 was 1s, the 90°[^1H] pulse was 9 μs. A total of 8 transients were collected for each t_1 values; the number of increments was 128

and the spectral width in the F_2 domain (3000 Hz) was twice the one in the F_1 domain. The data matrix was 256 x 512 data points and a sine bell window fonction was applied in both domains. Heteronuclear shift correlated NMR spectra [14] were obtained by using the pulse sequence described in the Bruker program XHCORRD.AUR. The spectral widths in $F_2(^{13}C)$ and in $F_1(^1H)$ were respectively 16000 Hz and 1500 Hz. The data were acquired using a matrix of 512 x 4096 points. The fixed delays correspond to a $^1J_{C,H}$ coupling constant of 140 Hz. A relaxation delay of 1s was used and 32 scans per increment were collected (number of increments = 256). Optical rotations were obtained on a Perkin Elmer 241 polarimeter. Mass spectra were performed on a Ribermag 10-10 (EI or CI NH_3). M.p.s were measured with a Büchi SMP-20 capillary melting point apparatus. Combustion analysis were performed by the C.N.R.S.(Vernaison). Analytical HPLC was carried out with a LKB 2249 metering pump model. The detection was performed with a HP 1040 Photodiode Array Spectrophotometer connected to a HP 9000 S300 Computer. TLC was performed on Merck pre-coated plates (Kieselgel F254) with the following solvent system : hexane-EtOAc (1:4, v/v).

General Procedure for the Synthesis of (3*RS*) 4-(*tert*-butoxycarbonylamino)-3-substituted-Butanoic Acids [(3*RS*)-2]

A mixture of di-*tert* -butyl carbonate(0.240 g, 1.1 mmol) in dioxane/water (2:1, v/v, 10 ml) is added dropwise to a stirred solution of (*RS*)-1 (0.214 g, 1 mmol) in dioxane/water (2:1, v/v, 10 ml) at such a rate to maintain the pH at 9 by careful addition of 1N aqueous NaOH. Stirring is maintained overnight. The solvent is evaporated and the residual oil is taken up with water. The solution is washed with Et_2O, acidified with citric acid (to pH 3) and extracted with EtOAc. The organic layer is dried over Na_2SO_4 and the solvent is evaporated *in vacuum* . The colorless

residual oil crystallises from hexane as a white powder. Compound **2 e** displayed the following $\nu_{max.}$ (KBr) 3000-2500 ((NH, OH) 1690 (CO Boc, CONH) cm^{-1}; δ_H (80 MHz, $CDCl_3$) 1.38 (9H, s, $C(CH_3)_3$), 2.50-2.70 (2H, m, CH_2CO), 3.00-3.40 (3H, m, CH_2N, CH), 3.82(3H, s, CH_3O), 4.70 (1H, br s, NH), 6.00 (1H, br s, OH), 6.46 (1H, s, H3), 6.84 (1H, dd, J46=2.5 and J76=8.0, H6), 6.93 (1H, d, H4), 7.31 (1H, d, H7); m/z 242,240(M^+-$OC(CH_3)_3$, 4.8, 0.8%) 224,222(3.3, 0.8) 198,196(34.7, 11.7); Rf: 0.37

General Procedure for the Synthesis of (3*RS*,8*S*) *N*-methylbenzyl-4-(*tert*-butoxycarbonylamino)-3-substituted-Butyramides [(3*RS*,8*S*)-3]

A mixture of (3*RS*)-**2** (0.219 g, 0.7 mmol) in THF (15 ml) with Et_3N (0.1 ml, 0.7 mmol) is cooled at -15°C. $ClCO_2Et$ (0.07 ml, 0.7 mmol) is added dropwise under intensive stirring. After a further 15 mn, (-)-(*S*) methyl benzyl amine (0.09 ml, 0.7 mmol) in THF solution is added dropwise to the reaction mixture. The temperature is maintained at -15°C for two hours. The solution is allowed to warm at room temperature and stirring is maintained overnight. The Et_3N,HCl precipitated is filtered off. The filtrate is evaporated *in vacuum*. The rough glassy is resolved in its two component by chromatography (HPLC).

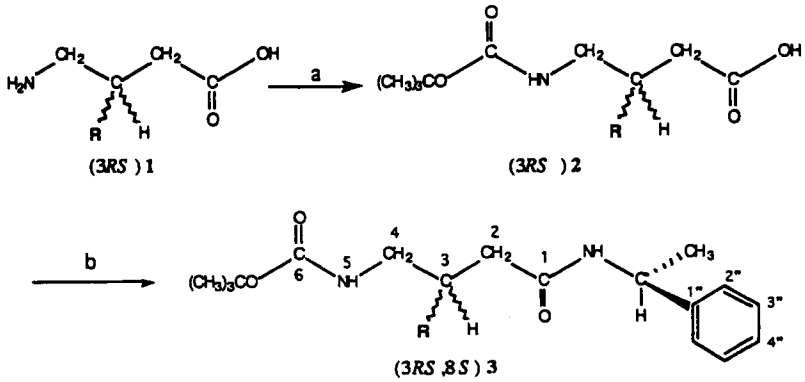
Compound **3 e** displayed the following $\nu_{max.}$ (KBr) **A** 3360 (NH) 1695, 1650 (CONH, CO Boc); **B** 3360, 3320 (NH) 1695, 1655 (CONH, CO Boc) cm^{-1}; m/z 343(M^+-$OC(CH_3)_3$, 0.8)301,299(5.1, 1.8)289,287(22.8, 7.6); Rf: 0.53(**A**) and 0.47(**B**); $[\alpha]^{22}_D$(**A**) = -33.8; $[\alpha]^{22}_D$(**B**) = +5.1 (c = 1.?90; $CHCl_3$)

RESULTS AND DISCUSSION

Various NMR techniques have been employed for the determination of enantiomeric purity using chiral solvating agents (c.s.a.), chiral lanthanides shift reagents (c.l.s.r.) and chiral derivatizing agents (c.d.a.) [18,17]. The basis of this NMR method involves conversion of a mixture of enantiomers into a diastereomeric mixture by reaction with an enantiomerically pure chiral reagent under non-racemising conditions. The diastereomeric ratio may be determined by direct integration of anisochronous resonances in the NMR spectrum of the diastereomeric mixture. In the present work, chiral derivatization was used for NMR techniques and correlated to HPLC.

Compounds **1** were prepared as previously described [10,20,22]. Racemates **1** were resolved into the enantiomers by a two-steps derivatisation and a fractionnal preparative HPLC (FIG. 1). Derivative **2** was obtained by treatment of **1** with di-*tert*-butyl dicarbonate to protect the amino group. Reaction of **2** with (*S*)-(-)-α-methylbenzylamine following the mixed anhydride method [11] furnish the 1:1 diastereomeric mixture **3**. The two diastereomers were readily distinguished by ^1H and analytical HPLC. Each separated component obtained after preparative chromatography was characterized by ^1H and ^{13}C NMR, anisochronous resonances being used as probes for quantitation and absolute structural assignment, purity being determined by analytical HPLC. The designation of **A** and **B** were chosen on the basis of the R_f and t_r values : **A** being the faster eluted product.

The ^1H NMR data for **3** are different for each diastereomer and are listed in Table 1. The GABA chain can be analyzed as a double ABX system. The 300



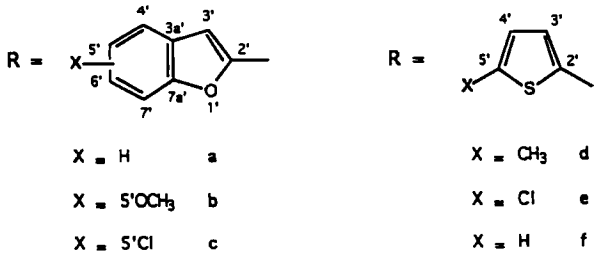


FIG. 1 Reaction scheme

MHz ^1H spectra of the aliphatic region of each compound show three well separated resonance ranges. We see a pattern of both eight lines for H2A, H2B and H4A', H4B' methylene protons respectively. A more complex multiplet can be attributed to H3X methine proton. The spectrum can be described as an ABX system on one hand and an A'B'X system on the other one. The most significant signal to distinguish them in the racemate mixture is the highfield part of the

TABLE 1

^1H NMR spectral data (p.p.m. from $SiMe_4$ in $CDCl_3$ as solvent at 300 MHz) for compounds **3**

Compd	H(7') CONH	H(2) H(2"); H(3"); H(4") $COCH_2$	H(3) CH	H(4) CH_2N	NHCOO	$CHCH_3$	$CHCH_3$	$C(CH_3)_3$	H(3')	H(4')	H(5')	H(6')
3a A	6.62 (b s)	2.67	3.52 (m)		4.83 (b s)	5.07 (q5) J=7.2	1.36 (d) J=6.8	1.44 (s)	6.53 (s)			7.18 - 7.56 (m)
3a B	6.60 (b s) J=7.1	2.66	3.52 (m)		4.82 (b s)	5.10 (q5) J=7.2	1.49 (d) J=7.0	1.45 (s)	6.50 (s)			7.20 - 7.58 (m)
$\Delta\delta_{AB}$					+4.0	-8.6	-35.1	-2.0	+9.2			

Compd	H(7') CONH	H(2) H(2"); H(3"); H(4") $COCH_2$	H(3) CH	H(4) CH_2N	NHCOO	$CHCH_3$	$CHCH_3$	$C(CH_3)_3$	CH_3O	H(3')	H(4')	H(5')	H(6')
3bA	6.50 7.32 (d) J=7.1	2.63 (m)	3.53 7.28 (m)		4.83 (b s)	5.07 (q5) J=7.3	1.36 (d) J=6.8	1.44 (s)	3.87 (s)	6.46 (s)	7.00 (d) J=2.5	-- --	6.88 (dd) (d) J=8.9
3bB	6.56 7.30 (d) J=7.1	2.62 (m)	3.53 7.20 (m)		4.83 (b s)	5.10 (q5) J=7.3	1.47(d) (d) J=7.2	1.45 (s)	3.87 (s)	6.42 (s)	6.98 (d) J=2.5		6.88 (dd) (d) J=8.9
$\Delta\delta_{AB}$	+4.9				-1.9	-8.3	-34.8	-1.9	-0.7	+11.9	+5.3		+1.0

3cA	6.42 7.22 - 7.26 (b s) (m)	2.59 (d)	3.50 (m)	4.85 (b s)	5.03 (q5) J=7.2	1.32 (d) J=6.8	1.39 (s)	6.42 (s)	7.44 (d)	--	7.18 - 7.28 (m)
3cB	6.56 7.11 - 7.19 (b s) (m) J=7.1	2.63 (d)	3.50 (m)	4.84 (b s)	5.03 (q5) J=7.2	1.43 (d) J=7.0	1.40 (s)	6.37 (s)	7.41 (d)	--	7.17 - 7.25 (m)
$\Delta\delta_{AB}$				+1.2	0	-33.1	-3.0	+15.1			

Compd	CONH	H(2) $COCH_2$	H(3) CH	H(4) CH_2N	NHCOO	$CH\underline{CH}_3$	$CH\underline{CH}_3$	$C(CH_3)_3$	H(3')	H(4')	H(2"); H(3"); H(4")		$(CH_3)_{5'}$
3dA	6.37 (b s)	2.52	3.52	3.38	4.79 (b s)	5.07 (q5) J=7.3	1.38(s) J=6.9	1.46(s)	6.64 (d d) J=1.0	6.60 (d) J=3.4	7.30 (m)		2.46(d) J=0.9
3dB	6.37 (b s)	2.51	3.56	3.42	4.79 (b s)	5.09 (q5) J=6.5	1.48(s) J=7.5	1.46(s)	6.62 (d d) J=1.0	6.58 (d) J=3.4	7.30; (m)	7.18(d) J=6.7	2.45(d) J=0.9
$\Delta\delta_{AB}$					+1.4	-7.2	-27.0	-2.1	+6.2	+6.4			+3.2

(continued)

TABLE 1

(continued)

Compd	CONH	H(2) $COCH_2$	H(3) CH	H(4) CH_2N	NHCOO	CH_2CH_3	$CHCH_3$	$C(CH_3)_3$	H(3')	H(4')	H(2"); H(3"); H(4")
3eA	6.47 (b s)	2.50	3.51	3.36	4.82 (b s)	5.07 (q5) J=7.3	1.42(s) J=7.3	1.46(s)	6.76 (d) J=3.7	6.73 (d)	7.30; 7.33; 7.26 (m)
3eB	6.45 (b s)	2.49	3.56	3.41	4.82 (b s)	5.09 (q5) J=7.2	1.48(s) J=6.8	1.46(s)	6.72 (d d) J=1.0	6.60 (d) J=3.4	7.30; 7.18(d) (m) J=6.7
$\Delta\delta_{AB}$					+0.1	-3.9	-18.2	-1.4	+10.9	+10.0	

Compd	CONH	H(2) $COCH_2$	H(3) CH	H(4) CH_2N	NHCOO	CH_2CH_3	$CHCH_3$	$C(CH_3)_3$	H(3'); H(4'); H(5')	H(2"); H(3"); H(4")
3fA	6.71 (b s)	2.54	3.56	3.33	4.89 (b s)	5.00 (q5) J=7.3	1.31(s) J=7.3	1.41(s)	6.82; 6.92; 7.15 (d) J=3.7	7.23; 7.27; 7.30 (m)
3fB	6.78 (b s)	2.56	3.62	3.39	4.93 (b s)	5.02 (q5) J=7.2	1.41(s) J=6.8	1.42(s)	6.78; 6.90; 7.14 (d) J=3.4	7.11; 7.20; 7.24 (m)
$\Delta\delta_{AB}$					-9.0	-5.9	-28.2	-1.9	+6.9	+3.0

$\Delta\delta$ is defined as the change in shift is from A to B (δ_A-δ_B) and is expressed in Hz

[a] s = singlet ; d = doublet ; t = triplet ; m = multiplet; q5 = quintuplet; b s = broad singlet

TABLE 2

^{13}C NMR spectral data (p.p.m. from $SiMe_4$ in $CDCl_3$ as solvent at 75 MHz) for compounds **3**

Compd	C(1) CONH	C(2) $\underline{C}H_2CO$	C(3) CH	C(4) CH_2N	C(6) NHCOO	$C(\underline{C}H_3)_3$	$\underline{C}(CH_3)_3$	C(8) $\underline{C}H(CH_3)$	$CH(\underline{C}H_3)$
3aA	169.86	37.79	37.43	42.62	156.46	28.33	79.86	49.16	21.73
3aB	169.83	37.84	37.48	42.58	156.47	28.35	79.84	48.98	21.65
$\Delta\delta_{AB}$	+2.3	-3.8	-3.8	+3.0	-0.8	-1.6	-1.6	+13.6	+6.0

	Aromatics							
	C(2')	C(3')	C(3a')	C(4')	C(5')	C(6')	C(7')	C(7a')	C(1");C(2");C(3");C(4")
3aA	154.69	103.84	128.40	120.81	122.87	123.91	110.98	157.79	143.06;126.23;128.64;127.33
3aB	154.71	103.86	128.53	120.82	122.84	123.90	111.01	157.77	142.98;126.04;128.53;127.20
$\Delta\delta_{AB}$	-1.6	-1.6	+4.0	-0.8	+2.3	+0.8	-2.3	+1.6	+6.0;+14.3;+8.3;+10.6

Compd	C(1) CONH	C(2) $\underline{C}H_2CO$	C(3) CH	C(4) CH_2N	C(6) NHCOO	$C(\underline{C}H_3)_3$	$\underline{C}(CH_3)_3$	C(8) $\underline{C}H(CH_3)$	$CH(\underline{C}H_3)$
3bA	169.83	37.82	37.51	42.65	156.43	28.35	79.83	49.14	21.75
3bB	169.88	37.80	37.53	42.58	156.48	28.36	79.83	49.01	21.82
$\Delta\delta_{AB}$	-3.8	+1.6	-1.6	+5.3	-3.8	-0.8	-	+9.8	-5.3

	Aromatics		
	OCH_3	C(2'); C(3'); C(3a'); C(4'); C(5'); C(6'); C(7'); C(7a')	C(1");C(2");C(3");C(4")
3bA	55.97	156.01; 104.00; 128.98; 103.40; 149.65; 112.43; 111.37; 158.64	143.07;126.23;128.63;127.33
3bB	55.99	155.99; 104.03; 128.97; 103.43; 149.67; 112.43; 111.40; 158.61	142.98;126.06;128.53;127.20
$\Delta\delta_{AB}$	-1.6	+1.6; -2.3; +0.8; -2.3; -1.6; 0; -2.3; +2.3	+6.8;+12.8;+7.5;+9.8

TABLE 2

(continued)

Compd	C(1) CONH	C(2) $\underline{C}H_2CO$	C(3) CH	C(4) CH_2N	C(6) NHCOO	$C(\underline{C}H_3)_3$	$\underline{C}(CH_3)_3$	C(8) $\underline{C}H(CH_3)$	$CH(\underline{C}H_3)$
3cA	169.52	37.69	37.40	42.60	159.66	28.30	79.79	49.02	21.73
3cB	169.62	37.71	37.49	42.62	156.52	28.32	79.83	48.95	21.72
$\Delta\delta_{AB}$	+7.3	-1.6	-6.5	-2.5	+12.1	-2.2	-3.2	+9.0	+1.1

	Aromatics							
	C(?')	C(3')	C(3a')	C(4')	C(5')	C(6')	C(7')	C(7a')	C(1");C(2");C(3");C(4")
3cA	156.34	103.29	129.79	120.32	128.37	123.98	111.85	158.64	143.08;126.16;128.59;127.29
3cB	156.38	103.44	129.77	120.35	128.35	123.97	111.87	158.61	142.96;125.99;128.49;127.22
$\Delta\delta_{AB}$	-3.1	-9.6	+1.5	-2.5	+2.0	+0.5	-2.3	+2.9	+13.0;+16.5;+11.1;+8.8

Compd	C(1) CONH	C(2) $\underline{C}H_2CO$	C(3) CH	C(4) CH_2N	C(6) NHCOO	$C(\underline{C}H_3)_3$	$\underline{C}(CH_3)_3$	C(8) $\underline{C}H(CH_3)$	$CH(\underline{C}H_3)$
3dA	169.93	41.49	38.98	45.78	156.32	28.39	79.63	48.93	21.78
3dB	169.94	41.57	39.04	45.71	156.36	28.40	79.63	48.80	21.82
$\Delta\delta_{AB}$	-0.8	-9.1	-4.5	+5.3	-3.0	-0.8	-	+9.8	-3.0

	Aromatics								
	$(CH_3)_5$'	C(2')	C(3')	C(4')	C(5')	C(1")	C(2")	C(3")	C(4")
3dA	15.33	143.28	124.92	124.74	142.53	138.43	126.25	128.59	127.25
3dB	15.33	143.19	124.94	124.77	142.49	138.40	126.13	128.51	127.13
$\Delta\delta_{AB}$	-	+6.8	-1.5	-2.5	-3.0	+2.3	+9.1	+6.0	+9.1

3eA	169.47	41.05	39.22	45.40	156.32	28.37	79.82	49.02	21.79	
3eB	169.54	41.21	39.39	45.45	156.33	28.38	79.84	48.89	21.78	
$\Delta\delta_{AB}$	-5.4	-11.8	-13.0	-5.3	-0.8	-1.1	-1.9	+10.3	+0.5	
					Aromatics					
		C(2')	C(3')	C(4')	C(5')		C(1")	C(2")	C(3")	C(4")
3eA		143.17	125.87	124.25	128.18		143.78	126.25	128.64	127.33
3eB		143.06	125.92	124.40	128.18		143.65	126.06	128.58	127.24
$\Delta\delta_{AB}$		+8.3	-4.1	-11.5	-0.5		+10.3	+14.3	+4.1	+9.0

Compd	C(1) CONH	C(2) $\underline{C}H_2CO$	C(3) CH	C(4) CH_2N	C(6) NHCOO	$C(\underline{C}H_3)_3$	$\underline{C}(C49)_3$	C(8) $\underline{C}H(CH_3)$	$CH(\underline{C}H_3)$	
3fA	169.98	41.37	38.66	45.82	156 29	28.36	79.49	48.85	21.77	
3fB	169.54	41.35	38.85	45.80	156.32	28.36	79.49	48.75	21.80	
$\Delta\delta_{Ab}$	-3.0	+1.5	-14.3	+1.5	-2.2	-	-	+7.5	-2.2	
					Aromatics					
		C(2')	C(3')	C(4')	C(5')		C(1")	C(2")	C(3")	C(4")
3fA		144.94	124.74	126.88	123.81		143.28	126.20	128.52	126.88
3fB		144.88	124.94	126.88	123.81		143.19	126.05	128.45	127.00
$\Delta\delta_{AB}$		+4.3	-4.3	0	0		+6.8	+11.3	+5.3	-9.0

$\Delta\delta$ is defined as the change in shift is from A to B (δ_A-δ_B) and is expressed in Hz

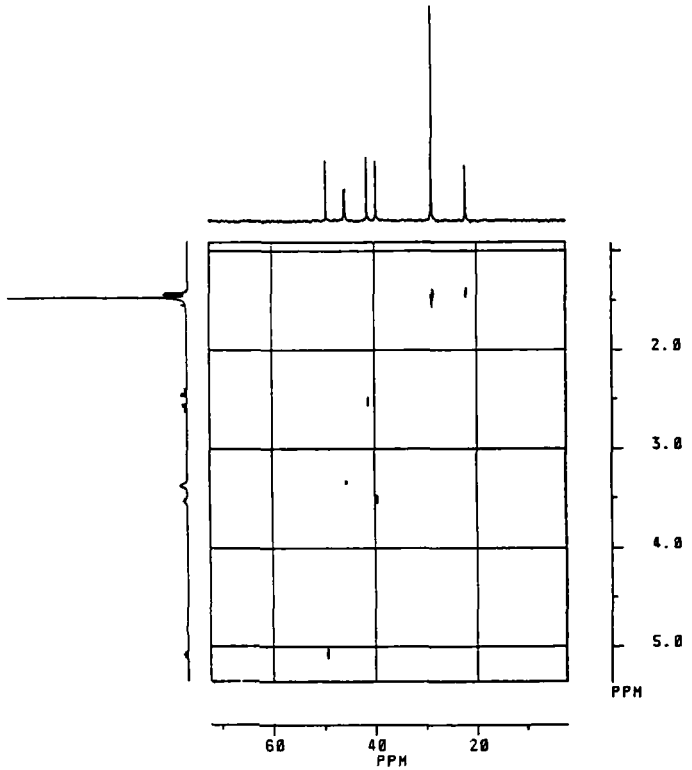


FIG. 2 The ^{1}H-^{13}C correlation NMR spectrum of **3eB** in the aliphatic region and in the aromatic region

spectrum with the three protons methyl doublet NCH(CH_3) (**3aA**, **3aB** : δ=1.38, 1.48; **3bA**, **3bB** : δ =1.42, 1.48; **3cA**, **3cB** : δ =1.31, 1.41; **3dA**, **3dB** : δ =1.38, 1.48; **3eA**, **3eB** : δ =1.42, 1.48; **3fA**, **3fB** : δ =1.31, 1.41 ppm) corresponding to a mean shift value of Δδ=0.10 ppm. The H3' proton of the benzofuranyl or thienyl ring near the chiral center of the GABA chain shows also significant shifts (**3aA**, **3aB** : δ=6.53, 6.50; **3bA**, **3bB** : δ =6.46, 6.42; **3cA**, **3cB** : δ =6.42, 6.37; **3dA**,

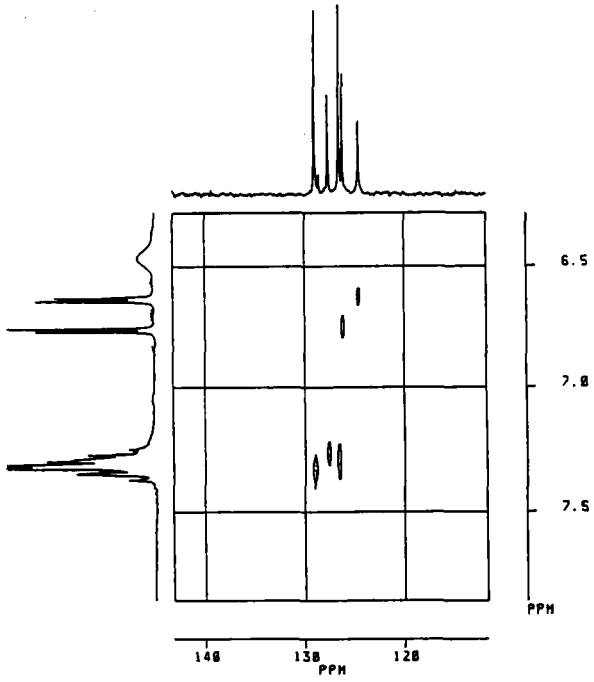
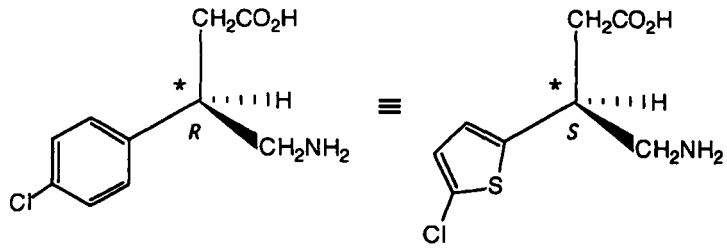


FIG. 2. Continued

3dB : δ =6.64, 6.62; **3eA**, **3eB** : δ =6.76, 6.72; **3fA**, **3fB** : δ =6.82, 6.78 ppm) corresponding to a mean shift value of $\Delta\delta$=0.04 ppm

Despite its low sensitivity the advantages of using ^{13}C NMR are clear when the proton NMR spectra are too complex to resolve resonances : for exemple with a mixture. The ^{13}C NMR spectra of **3** present appreciable differences and their data are summarized in Tables 2. The easiest assignments, in the racemates mixtures, to characterize each diastereomer are performed with the shifts of the GABA (N-CH_2-CH-CH_2-C0; C(2)-C(3)-C(4)) chain (**3a** : $\Delta\delta_{AB}$ = -0.08, -0.06, +0.07; **3b** :



baclofen 1 b

FIG. 3 The absolute configuration of **1 b** compared to baclofen

$\Delta\delta_{AB}$ = -0.16, -0.17, -0.05; **3 c** : $\Delta\delta_{AB}$ = +0.02, -0.19, +0.02; **3 d** : $\Delta\delta_{AB}$ =-0.08, -0.06, +0.07; **3 e** : $\Delta\delta_{AB}$ = -0.16, -0.17, -0.05; **3 f** : $\Delta\delta_{AB}$ = +0.02, -0.19, +0.02 ppm) and particularly the C3 atom. The $\underline{C}H(CH_3)_3$ carbon (**3 a, 3 b, 3 c, 3 d, 3 e, 3 f** : $\Delta\delta_{AB}$ = +0.08, +0.13, +0.10, +0.13, +0.13, +0.10) seems also interesting to characterize the compounds.The carbons of the phenyl ring (C1", C2", C3", C4") are all affected by the neighbourhood of the chiral center and particularly the C2" atom (**3 a** : $\Delta\delta_{AB}$ = +0.19; **3 b** : $\Delta\delta_{AB}$ = +0.17; **3 c** : $\Delta\delta_{AB}$ = +0.17; **3 d** : $\Delta\delta_{AB}$ +0.12; **3 e** : $\Delta\delta_{AB}$ = +0.19; **3 f** : $\Delta\delta_{AB}$ = +0.15 ppm)

Complete and unambiguous assignments were made following the differents techniques [12-14] (^1H -^{13}C correlation NMR spectrum - FIG. 2) and corroborated with literature data [7,9,15,16]. The **A/B** ratios obtained by HPLC and NMR (^1H and ^{13}C) are similar and were found to be 1:1. Following the Cahn-Ingold-Prelog rules i) for baclofen the substituent order is CH_2N > phenyl > CH_2CO > H ; but ii) for **1**,**2**,**3** molecules, this order becomes benzofuranyl or thienyl > CH_2N > CH_2CO > H. So for an identical spatial structure the nomenclature is reversed when we compare our molecules to baclofen (FIG.3).

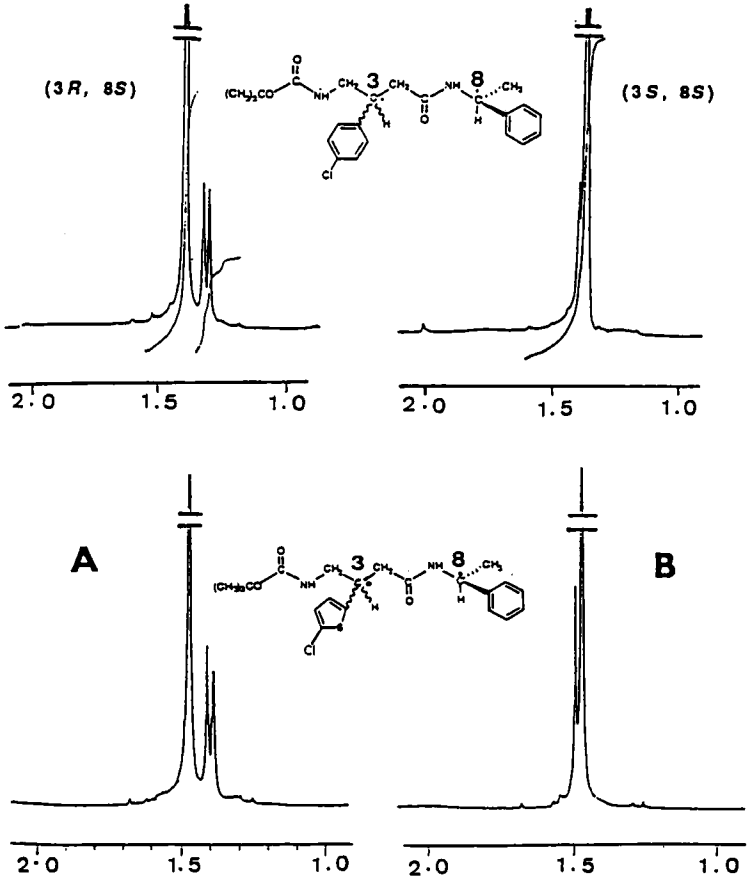


FIG. 4 Comparison of the lower part of ^{1}H NMR spectrum of baclofen diastereomers and of **3 e** diastereomers

The absolute configuration (*3S8S*) and (*3R8S*) were assigned to **A** and **B** on the basis of the corresponding behavior of the diastereomers obtained, through the same synthetic route, from the pure available enantiomers of baclofen whose absolute stereochemistry is known by crystallography. The same relative behavior was observed both in ^{1}H and ^{13}C NMR, HPLC and TLC. In HPLC for baclofen the (*3R8S*) diastereomer is eluted prior the (*3S8S*) diastereomer (FIG.4).

NMR spectroscopy and HPLC analysis make possible to determine the absolute stereochemistry of the two isomers of **1** unequivocally and to quantify the isomer ratio, without measuring the optical rotation. In conclusion the NMR configurational correlation associated with the HPLC elution order seems to be a highly useful method for the determination of the absolute configuration and separation of various γ-amino acids isomers.

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