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Complete ^{1}H and ^{13}C NMR spectral assignment of benzo[*d*]isothiazole derivatives and of an isoindole isoster

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The complete ^{1}H and ^{13}C NMR assignments of the novel compound methyl 2-amino-3-(benzo[*d*]isothiazol-3-yl)propanoate (1), of 3-amino-5-methylbenzo[*d*]isothiazole (2) and *N*-(*t*-butyloxycarbonyl)-2-aminobenzo[*d*]isothiazol-3(2*H*)-one (3) and of the desulfurated isostere of 3, *N*-(*t*-butyloxycarbonyl)-2-aminoisoindolin-1-one (4), using 1D and 2D NMR techniques, including COSY, INADEQUATE, HSQC, and HMBC experiments are reported.

Keywords: 2D NMR; ^{1}H–^{13}C long-range coupling; HSQC; HMBC; INADEQUATE, 3-substituted benzo[*d*]isothiazole; 2-aminobenzo[*d*]isothiazol-3-one; 2-aminoisoindolin-1-one

Introduction

The benzo[*d*]isothiazole derivatives are heterocyclic compounds that have been reported to possess many interesting biological activities.[1,2] The benzo[*d*]isothiazole ring can be a substituent of a bioactive scaffold or the pharmacophore of a bioactive molecule. For a number of years, we have designed, synthetized and tested series of 3-substituted benzisothiazole derivatives[3–10] and more recently, we have discovered novel 2-aminobenzo[*d*]isothiazole-3-one derivatives displaying new, interesting biological properties.[11–14] Although a lot of structural data have been reported in the literature concerning the chemistry of the benzofused isothiazoles,[1,2,15] detailed information about ^{1}H and ^{13}C NMR signals of the class of compounds is still lacking. In pursuing studies on this class of benzisothiazole derivatives, structural elucidation is a significant part of our work. So, we decided to perform the complete ^{1}H and ^{13}C NMR assignment of a novel 3-substituted derivative (**1**) and of the previously described 3-amino-5-methylbenzo[*d*]isothiazole (**2**), the parent compound of 5-methyl substituted benzo[*d*]isothiazoles extensively investigated by us (Table 1).[3,7–9,16] Moreover, we report here the complete spectral assignment of the key intermediate (**3**) used for the synthesis of 2-aminobenzo[*d*]isothiazol-3-one derivatives[11–14] and of its desulfurated isoster, the 2-aminoisoindolin-1-one analog (**4**) (Table 2).

Results and Discussion

All ^{1}H and ^{13}C chemical shifts and coupling constants are given in Tables 1 and 2 along with the HMBC ^{13}C–^{1}H correlations on which the unambiguous assignments of all carbons and protons were made. The structures of the compounds **1**–**4** were assigned by analysis of one- and two-dimensional NMR experiments.

In the case of **1** (Table 1), the amino protons and the protons on the carbons 4, 5, 6, 7, 8, 9, and 11, which were easily assigned on the basis of chemical shift and multiplicity, allowed the assignment of the carbons they are directly attached to, by C–H correlation (gHSQC). The gCOSY spectra allowed assignment of the aromatic spin system pattern H4-H5-H6-H7, but the two couples H4–H7 and H5–H6 might be interchanged. In principle, H4 and H7 can be discriminated on the basis of C–H correlations governed by $^{2}J_{CH}$ and $^{3}J_{CH}$. In gHMBC spectra, H4 proton should show long-distance $^{3}J_{CH}$ correlations with C6 and with the two quaternary carbons C3 and C7a. On the other hand, H7 must show the long-distance $^{2}J_{CH}$ correlations with C6 and C7a and $^{3}J_{CH}$ correlations with C3a and C5, but not with C3. In fact, supposing H4 at $\delta = 7.95$ ppm, in addition to the $^{2}J_{CH}$ correlation with C3a, we found, for H4, the $^{3}J_{CH}$ correlations with C6 and three quaternary carbons at $\delta = 162.6$, 134.8, and 152.4 ppm; for H7 we found the $^{2}J_{CH}$ correlation with C6 and the $^{3}J_{CH}$ correlations with C5 and a quaternary carbon at $\delta = 134.8$ ppm. This suggests that C3, C3a, and C7a should be at $\delta = 162.6$, 134.8, and 152.4 ppm, respectively. Both H4 and H7 showed a mutual weak correlation. H5 showed the expected $^{2}J_{CH}$ and $^{3}J_{CH}$ correlations with C3a, C4, C6, and C7 together with two $^{4}J_{CH}$ correlations with C3 and C7a. H6 showed exactly the expected correlations with C4, C5, C7, and C7a.

The assignment of **2** was easier (Table 1); the signal of H4 was a singlet, which showed a $^{2}J_{CH}$ coupling with C3a and a number of long-range $^{3}J_{CH}$ correlations with C3, C6, C7a, and with the methyl carbon C8.

For compound **3** (Table 2), the protons and carbon atoms 4, 5, 6, 7, and 10 were assigned using the same strategy as described for **1**; the HMBC experiment showed that H4 had strong correlations with C6, and with three quaternary carbons at $\delta = 122.0$, 140.4, and 164.9 ppm, while H7 showed the expected $^{3}J_{CH}$ correlations

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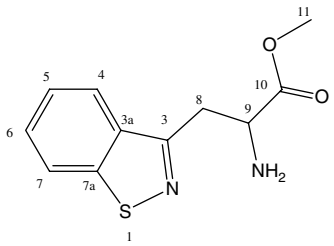
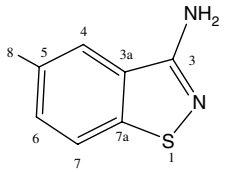
Table 1. ^{1}H and ^{13}C chemical shifts[a] and HMBC correlations[b] of **1** and **2**





	1				2		
Position	^{1}H	^{13}C	HMBC	Position	^{1}H	^{13}C	HMBC
3	–	162.6	–	3	–	158.3	–
3a	–	134.8	–	3a	–	126.7	–
4	7.95 (d, $J = 8.3$)	123.1	3,3a,6,7,7a	4	7.45(s)	121.3	3,3a,6,7a,8
5	7.42 (t, $J = 8.3$)	124.7	3,3a,4,6,7,7a	5	–	134.0	–
6	7.50 (t, $J = 8.3$)	127.7	4,5,7,7a	6	7.30 (d, $J = 8.2$)	129.9	4,5,7,7a,8
7	7.90 (d, $J = 8.3$)	119.9	3a,4,5,6	7	7.65 (d, $J = 8.2$)	120.0	3a,4,5,6
7a	–	152.4	–	7a	–	149.1	–
8a	3.41 (dd, $J_{8a-9} = 7.8$, $J_{8a-8b} = 15.4$)	36.1	3,3a,9,10	8	2.45 (s)	21.2	4,5,6
8b	3.54 (dd, $J_{8b-9} = 4.7$, $J_{8a-8b} = 15.4$)	–	3,3a,9,10	NH_2	4.81 (b)	–	–
9	4.14 (dd, $J_{9-8a} = 7.8$, $J_{9-8b} = 4.7$)	53.3	3,8,10	–	–	–	–
10	–	175.1	–	–	–	–	–
11	3.69 (s)	52.2	10	–	–	–	–
NH_2	1.87 (s)	–	–	–	–	–	–

[a] Chemical shifts in ppm (multiplicity, J in Hz).
[b] Carbons coupled to the corresponding H atom.

with C5 and with the quaternary carbon at $\delta = 122.0$ ppm. Both H4 and H7 showed mutual correlations due to $^{4}J_{CH}$; moreover, H7 showed a weak $^{4}J_{CH}$ correlation at $\delta = 164.9$. This suggests that the signals at $\delta = 164.9$ and 122.0 ppm belong to C3 and C3a, respectively. So, H4 showed a $^{2}J_{CH}$ correlation with C3a but a very weak coupling with C5; also, H7 showed only one $^{2}J_{CH}$ correlation with C6, but H7–C7a coupling was missing. Moreover, H5 and H6, besides the expected $^{2}J_{CH}$ and $^{3}J_{CH}$, showed the $^{4}J_{CH}$ couplings: H5–C3, H5–C7a, and H6–C3a. These anomalies in the intensities of the cross-peaks suggested us to run an INADEQUATE experiment to build a reliable C–C correlation map. This is a very powerful experiment; however, it is not very sensitive and needs a very concentrated sample solution to provide a C–C connectivity map.

Starting from the unambiguous C3 (Fig. 1), the only carbonyl group without proton correlation, it is easy to trace the aromatic ring correlation net; C3a correlates with C4, but has another connection with a carbon at $\delta = 140.4$ ppm, which cannot be other than C7a. This spectrum allows discriminating C3a from C7a.

The same approach was used for compound **4** (Table 2): the protons and carbon atoms 1, 4, 5, 6, 7, and 10 were assigned using the same strategy as described for **1**. Both gHMBC and INADEQUATE were used to assign all carbon atoms. In particular in gHMBC spectrum, the correlations of H1 with C7a and C7 are diagnostic to discriminate H4 from H7. So, starting from the unambiguous C4, it is easy to trace the aromatic ring correlation net; C3a has a second connection with a carbon at $\delta = 167.6$ ppm, which can be only C3.

Experimental

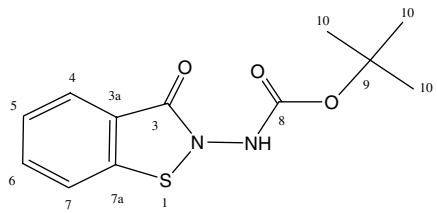
Synthesis

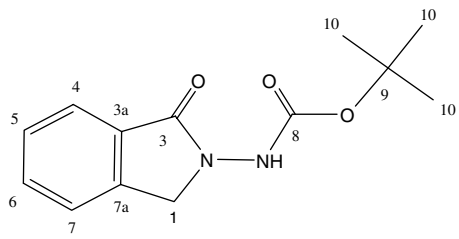
The synthesis of methyl 2-amino-3-(benzo[*d*]isothiazol-3-yl)propanoate (**1**) was accomplished as reported in Fig. 2. According to Uno and Kurokawa,[17] 3-(bromomethyl)benzo[*d*]isothiazole was obtained from 2-(benzo[*d*]isothiazol-3-yl)acetic acid through bromination under suitable conditions, followed by decarboxylation. It was converted, by reaction with diethyl 2-acetamidomalonate, to diethyl 2-acetamido-2-[(benzo[*d*]isothiazol-3-yl)methyl]malonate. Hydrolysis of the latter, in 6 M hydrochloric acid, afforded the hydrochloride salt of 2-amino-3-(benzo[*d*]isothiazol-3-yl)propanoic acid, which was easily converted to target **1**.

The synthesis of 3-amino-5-methylbenzo[*d*]isothiazole (**2**)[16] as well as that of *N*-(*t*-butyloxycarbonyl)-2-aminobenzo[*d*]isothiazol-3(2*H*)-one (**3**)[11] was described earlier. The preparation of the new *N*-(*t*-butyloxycarbonyl)-2-aminoisoindolin-1-one (**4**) was performed starting phthalide from which, according to Burton and Koppes,[18] was initially heated with dibromotriphenylphosphorane, to give 2-(bromomethyl)benzoylbromide, which was then treated with *tert*-butyl carbazate in anhydrous dioxane in the presence of triethylamine (TEA) to yield **4** (Fig. 3).

All reactions were carried out using flame-dried glassware under an atmosphere of nitrogen. Anhydrous dichloromethane was obtained by its distillation from dried granular calcium chloride. Melting points were measured on a Buchi 512 apparatus and are uncorrected. Flash chromatography was performed using Merck silica gel 60 (Si 60, 40–63 µm, 230–400 mesh ASTM).

Table 2. ^{1}H and ^{13}C chemical shifts[a] and HMBC correlations[b] of **3** and **4**





	3				4		
Position	^{1}H	^{13}C	HMBC	Position	^{1}H	^{13}C	HMBC
1	–	–	–	1	4.57 (s)	51.7	3,3a,6,7,7a
3	–	164.9	–	3	–	167.6	–
3a	–	122.0	–	3a	–	130.3	–
4	8.02 (d, $J = 8.2$)	127.3	3,3a,5,6,7a,7	4	7.82 (d, $J = 7.7$)	124.2	3,3a,6,7,7a
5	7.35 (t, $J = 8.2$)	125.5	3,3a,4,6,7,7a	5	7.41 (t, $J = 7.7$)	128.0	3,3a,4,6,7,7a
6	7.60 (t, $J = 8.2$)	132.8	3a,4,5,7,7a	6	7.53 (t, $J = 7.7$)	132.2	3a,4,5,7,7a
7	7.44 (d, $J = 8.2$)	120.4	3,3a,4,5,6	7	7.37 (d, $J = 7.7$)	122.9	1,3a,4,5,6,7a
7a	–	140.4	–	7a	–	140.3	–
8	–	153.8	–	8	–	154.7	–
9	–	83.0	–	9	–	82.0	–
10	1.45 (s)	28.0	9	10	1.44 (s)	28.1	9
NH	7.29 (b)	–	–	NH	7.17 (b)	–	–

[a] Chemical shifts in ppm (multiplicity, J in Hz).
[b] Carbons coupled to the corresponding H atom.

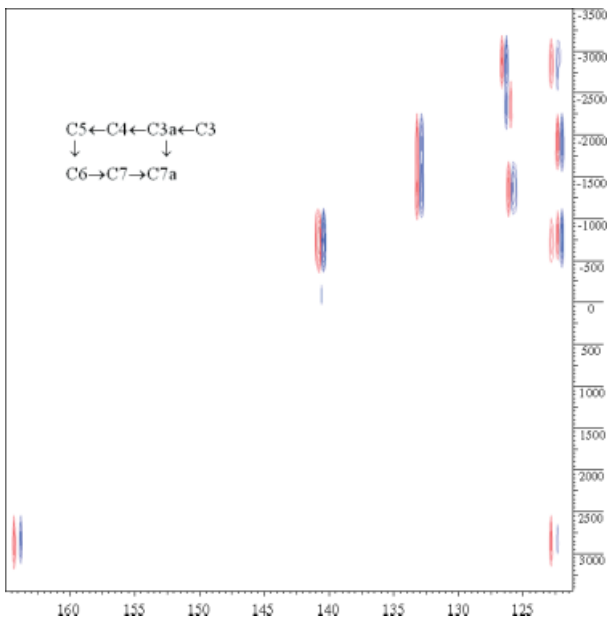


Figure 1. Compound **3**: INADEQUATE spectrum and carbon connectivities.

Elemental analyses for C, H, N, and S were performed using a ThermoQuest Flash 1112 Elemental Analyzer. IR spectra were recorded on a Jasco FT-IR 300E spectrometer. Mass spectra were recorded on an Applied Biosystem, API 150 EX LC/MS system spectrometer. NMR spectra were run on a Varian INOVA 600 spectrometer.

Methyl 2-amino-3-(benzo[d]isothiazol-3-yl)propanoate (1)

To an ethanolic suspension of sodium diethyl 2-acetamidomalonate[19] (10 mmol), a solution of 3-(bromomethyl)benzo[*d*]isothiazole (1.94 g, 8.5 mmol) in anhydrous CH_2Cl_2 was added dropwise and the reaction mixture was heated at 90 °C for 15 min. The solvent was then evaporated under reduced pressure and the residue, poured into water, was extracted with CH_2Cl_2. From the organic extract, dried over Na_2SO_4 and evaporated, a yellowish oil was obtained, which solidified slowly, and was collected after elution on silica gel, by flash chromatography (methylene chloride-ethanol: 98/2), to yield 1.62 g (1.7 mmol) of diethyl 2-acetamido-2-[(benzo[*d*]isothiazol-3-yl)methyl]malonate. Then, it was used as such, for the following reaction step; it was suspended in 6 N HCl (10 ml) and refluxed for 12 h. After cooling and evaporation of the solution under reduced pressure, the reddish solid residue, consisting of 2-amino-3-(benzo[*d*]isothiazol-3-yl)propanoic acid hydrochloride, was carefully dried and used as a crude compound for the following chlorination/esterification. To a cooled methanolic solution (20 ml) of the above amino acid hydrochloride (0.5 g, 1.9 mmol), $SOCl_2$ (0.8 ml, 11 mmol) was added. After 15 min at 0 °C, under stirring, the reaction mixture was warmed up to room temperature and then refluxed for 30 min. The residue, obtained by concentration of the solution *in vacuo*, was subjected to silica gel flash chromatography with CH_2Cl_2/MeOH/NH_3 (98 : 2 : 0.1) and CH_2Cl_2/MeOH/NH_3 (95 : 5 : 0.25), when the expected pure product was obtained as a pale yellow viscous oil, which became solid slowly and was recrystallized from ethanol/water. Yield 75%; mp 223–225 °C; IR (KBr) (ν_{max} cm^{-1}): 3230 and 3185 (NH_2), 3068 (CH Ar), 2937 (CH_3), 1677 (CO). MS (APCI) $m\,z^{-1} = 237$, $(M + 1)^+$. Calc.

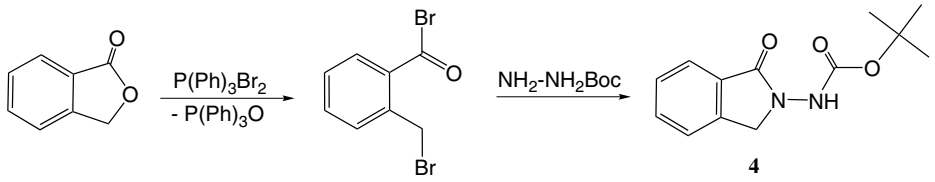


Figure 2. Synthesis of compound **1**.

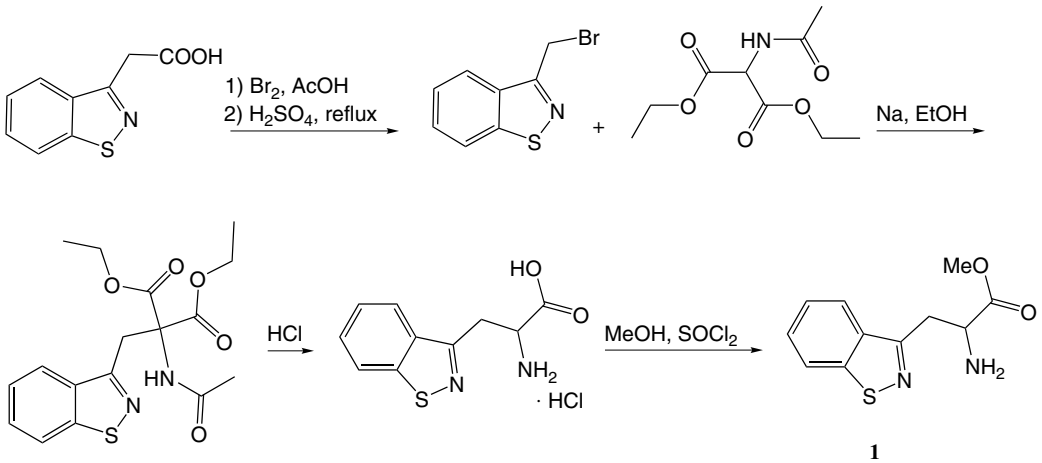


Figure 3. Synthesis of compound **4**.

for $C_{11}H_{12}N_2O_3S$: mz^{-1} = 236.29; C, 57.58; H, 5.64; N, 11,19; S, 12.81%. Found: C, 57.80; H, 5.27; N, 11.27, S, 12.65%.

3-Amino-5-methylbenzo[d]isothiazole (2) was prepared and purified as described elsewhere.[16]

N-(t-Butyloxycarbonyl)-2-aminobenzo[d]isothiazol-3(2H)-one (3) was prepared and purified as described earlier.[11]

N-(t-Butyloxycarbonyl)-2-aminoisoindolin-1-one (4)

To a magnetically stirred solution, containing triethylamine (2.5 ml) and 2-(bromomethyl)benzoylbromide (1.12 g, 4 mmol) in anhydrous dioxane (5 ml), *tert*-butylcarbazate (2.00 g, 15.2 mmol), dissolved in the same solvent (10 ml), was added dropwise. After refluxing for 2 h, the reaction mixture was cooled, and then the solvent was removed under reduced pressure. The residue was extracted with CH_2Cl_2, washed with 2 N HCl, and then dried over Na_2SO_4. The crude product was obtained after concentration under reduced pressure. By silica gel flash chromatography with CH_2Cl_2/MeOH/NH_3 (98 : 2 : 0.1), the expected pure product was separated and further purified by recrystallization from ethanol/water. Yield 27%; mp 173–175 °C; IR (KBr) (ν_{max} cm^{-1}): 3274 (NH), 2975 and 2929 (CH_3), 1731 and 1693 (CO). Anal. Calcd. for $C_{13}H_{16}N_2O_3$ (248.12): C, 62.89; H, 6.50; N, 11.28%. Found C, 63.01; H, 6.49; N, 11.06%.

NMR measurements

The structural and spectroscopic assignments were made through the combined use of 1 and 2D homonuclear NMR experiments, such as COSY and INADEQUATE, and heteronuclear HSQC and HMBC correlation techniques. ^{1}H and ^{13}C NMR, gCOSY, gHSQC, gHMBC spectra were obtained from samples as $CDCl_3$ solutions (50 mg ml^{-1}). INADEQUATE spectra were obtained from samples as DMSO solutions (500 mg ml^{-1}) and calibrated setting the DMSO residual signal at δ = 2.49 ppm.

The ^{1}H and ^{13}C NMR spectra (at δ = 599.74 and 150.82 MHz, respectively) were measured at 25 °C in 5 mm o.d. tubes. Chemical shifts are reported as δ (ppm); coupling constants (J) are expressed in Hz. Spectra were referenced to residual $CHCl_3$ at δ = 7.24 ppm. In 1D ^{1}H NMR analysis, 16 transients were acquired with a 1.5 s relaxation delay using 32K data points and zero-filled to a digital resolution of 0.17 Hz. The ^{13}C NMR spectra were run operating typically with a 45° pulse flip angle, a relaxation delay time of 3–5 s and a spectral width of 25 000 Hz with 64K data points and zero-filled to a digital resolution of 0.4 Hz.

The data for the gCOSY, gHSQC, and gHMBC spectra were collected with 2K data points for F_2 and 128–256 increments for F_1 with pulse sequences that allowed gradient selection; spectral windows were 5500–6000 and 27 000–37 000 Hz in the F_2 (^{1}H) and F_1 (^{13}C) dimensions, respectively. The relaxation delay was set to 1.5 s and evolution time was varied between 60 and 100 ms. Once acquired, the data were processed using sine-bell weighting functions in both dimensions; 2K data points were zero-filled to 4K in F_2 and 256 data were linear predicted to 768 data points in F_1. INADEQUATE was recorded with a recycle time of 3 s, a spectral width of 9050 Hz in F_2 and 18 100 in F_1, and 2K data points for 128 increments of 256 transients. J(^{13}C, ^{13}C) was set to 55 Hz.

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