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Derivatization of Aminoacids with Fluorine Containing Markers – a Way for Their Determination by ^{19}F NMR Spectroscopy

by P. Szczeciński* and D. Bartusik

Warsaw University of Technology, Faculty of Chemistry, Noakowskiego 3, 00-664 Warszawa, Poland

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A new, water soluble marker, **2**, suitable for analysis of aminoacid mixtures, as well as its derivatives **3** and **4** with several aminoacids have been prepared and characterized by NMR spectroscopy. Remarks on benzimidazole ring formation have been given.

Key words: ^{19}F NMR, aminoacids determination, benzimidazole oxides

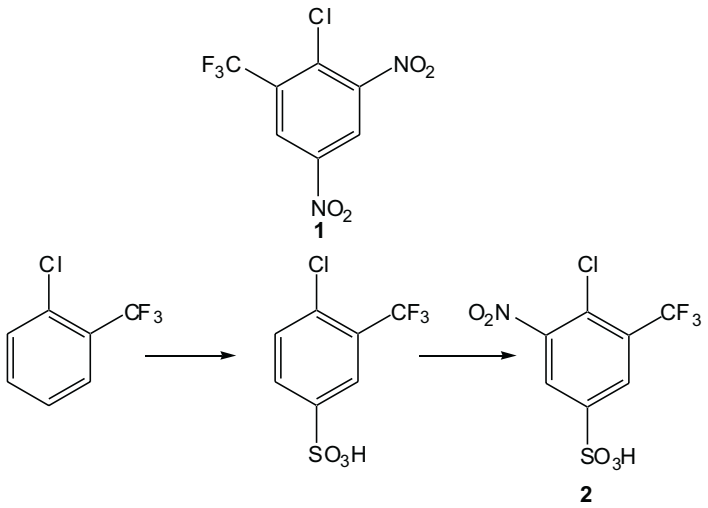
Looking for the method of qualitative and quantitative determination of aminoacids in complex mixtures, *e.g.* in body fluids, we paid our attention to the ^{19}F NMR spectroscopy. The use of this method in such investigations is profitable for several reasons. First of all there is no measurable amount of fluorine derivatives in living organisms which eliminates "chemical noise", characteristic for magnetic resonance spectra of proton or carbon-13 nuclei. Sensitivity of fluorine nuclei is similar to that of protons but the range of appearance of fluorine signals is about twenty times wider. Fluorine chemical shift is affected not only by electron influence of directly bonded atoms but also, and in remarkable extent, by the steric interactions. For these reasons the ^{19}F NMR measurements may be an useful tool for aminoacids determination, provided one is able to mark them selectively with the fluorine possessing group. In previous paper [1] we reported the use of several fluorine containing compounds as aminoacid markers, among which 2-chloro-1,5-dinitrotrifluoromethylbenzene, **1**, appeared to be most promising. It reacts with aminoacids to give appropriate *N*-substituted derivatives. Their CF_3 chemical shift values are spread over sufficiently wide range to use them for unequivocal aminoacid identification. The procedure elaborated has been successfully applied for the determination of several aminoacids in samples of urine and blood. Marker **1**, however, is insoluble in water. For this reason ethanol has to be added during aminoacid derivatization to assure the homogeneity of the reacting mixture. It was found that alcohol remarkably affects the fluorine chemical shift and therefore it must be thoroughly removed from the measured sample to assure the reproducibility of the results obtained. To avoid this inconvenience we decided to synthesise a marker, which, being water soluble, would still possess good points of compound **1**.

* Corresponding author. Fax: +48 22 628 27 41. *E-mail address:* przemsz@ch.pw.edu.pl

RESULTS AND DISCUSSION

4 Chloro-3-nitro-5-trifluoromethylbenzenesulfonic acid (**2**) was selected as a compound fulfilling demands defined above. The route of its synthesis is shown in Scheme 1.

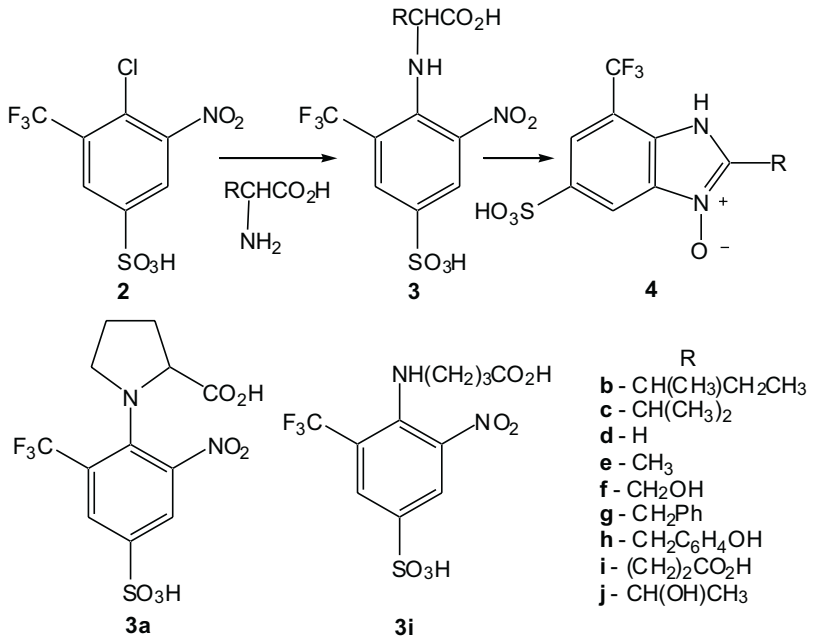
Scheme 1



Sulfonation of 1-chloro-2-trifluoromethylbenzene has been initially attempted at elevated temperature with the use of concentrated sulphuric acid. In consecutive experiments the mixtures of products were however obtained, among which the main ones were identified, on the basis of NMR spectra, as 2-chlorobenzoic acid and 2-chloro-5-sulfobenzoic acid. Good results were finally obtained when the procedure of sulfonation of 1-chloro-4-(trifluoromethyl)benzene [2] was adapted (65% oleum, temp. < 0°C). The conditions used in the second step of the synthesis were similar to those applied for nitration of 3-(trifluoromethyl)benzenesulfonic acid [3]. Besides of the main, desired product, a small amount of **1** was formed. Fortunately, it could be easily separated from **2** (see Experimental).

Marker **1** easily reacts with aminoacids in refluxed water-ethanol sodium bicarbonate solution giving aromatic nucleophilic substitution products, analogues of **3** [1]. In the case of compound **2** the same conditions for preparation of **3** were used but reaction was run in water. Surprisingly**,** benzimidazole derivatives **4**, instead of **3**, were this time obtained for several aminoacids used (see Scheme 2 and Table 1). Monitoring of the progress of the process by ^{19}F NMR spectra revealed that the cyclization run fast and product **3** did not constitute the main component of the mixture at neither stage of the reaction. When the reaction was performed at 70°C one could, after appropriate long time, obtain compound **3** as a main product but it was still accompanied by **2** and **4**. Moreover, prolonged time of the reaction increased amount of the product of CF_3 group hydrolysis [4,5], which was manifested by increased intensity of fluorine anion signal.

Scheme 2



An attempt of separation of **3** from such a mixture failed. Accidentally, it was found that the process of cyclization could be effectively stopped, when reaction was done at 100°C in closed vessel. The reason of this phenomenon is not clear for the present. The cyclization does not seem to be a reversible process. Heating of aqueous bicarbonate solution of **4e** at 100°C in carbon dioxide atmosphere under high pressure (60 at) did not give even a trace of **3e**. One may suspect that carbon dioxide, formed at aminoacids neutralization and during S_NAr reaction, being unable to escape, lowers basicity of the reacting mixture to the extent which precludes proton subtraction, the stage initiating cyclization process. Performing reaction in closed vessel compounds **3** were obtained for a few aminoacids (see Table 1).

Table 1. ^{19}F NMR chemical shifts of aminoacid derivatives of marker **2** in water.

Aminoacid	Compound	Derivative **3**	Derivative **4**
Pro	**a**	18.51	–
Ile	**b**	18.01	16.13
Val	**c**	17.70	16.18
Gly	**d**	18.39	16.21
Ala	**e**	17.23	16.39
Ser	**f**	17.73	16.45
Phe	**g**	18.92	16.51
Tyr	**h**	17.72	16.54
GABA	**i**		17.04
Thr	**j**		17.07

The cyclization of *N*- and arene ring substituted *o*-nitroanilines has been extensively investigated [6,7 and refs. therein]. To our knowledge the reactions proceeding under basic condition concerned compounds in which nitrogen atom was substituted with CH_2CO_2R [8–13], CH_2CN [8–11,13–16] or CH_2Ph [17]. Our previous investigations have revealed that the reaction do not demand the presence of negative charge stabilizing groups at the carbon atom which forms new C–N bond [6]. It proceeds smoothly for *N*-alkyl substituted 2,4-dinitro-6-trifluoromethylanilines (alkyl = CH_3, C_2H_5, $(CH_2)_3CH_3$, CH_2OH, $(CH_2)_3CO_2H$) and for *N*-ethyl-2,4,6-trinitroaniline. It was also found that this reaction does not run for *N,N*-dialkylanilines. This would indicate that the first step of cyclization is N-H proton subtraction. This probably facilitates the next step, in which, inevitable for ring closure carboanion is formed by C-H proton subtraction or, in the case of **3**, by loss of carbon dioxide molecule.

As it was mentioned above compounds analogous to **3** were easily prepared from marker **1** and appropriate aminoacids [1]. It was interesting to check if they could also be transformed into appropriate imidazole derivatives. It was found to be possible when reaction was run in ethanol with potassium carbonate as catalyst. Under this conditions for example *N*-(2,4-dinitro-6-trifluoromethylphenyl)-glycine, -alanine and -serine gave 5-nitro-7-trifluoromethyl-1*H*-benzimidazole 3-oxide and its 2-methyl and 2-hydroxymethyl derivatives, respectively. Obtained compounds were identical with those prepared previously from *N*-methyl-, *N*-ethyl- and *N*-(2-hydroxyethyl)-2,4-dinitro-6-trifluoromethylanilines [6].

The main goal of presented work was to check if aminoacids marked by compound **2** could be distinguished by ^{19}F NMR spectra. The chemical shift values of derivatives **3** and **4** in water (pH 8.5) collected in Table 1 spread, in both cases, over *ca*. 1 ppm range. Though one would wish this range to be wider it still seems to be proper for the use of new obtained marker **2** in analyses of aminoacid mixtures.

EXPERIMENTAL

^1H, ^{13}C and ^{19}F NMR spectra of aminoacid derivatives **3** and **4** (in DMSO-d_6) were recorded using a Varian Gemini 2000 spectrometer operating at 4.7 T. Residue solvent signals were used as chemical shift references for proton (δ_{DMSO} = 2.49 ppm) and carbon (δ_{DMSO} = 39.50 ppm), and $CFCl_3$ signal (δ_F = 0 ppm) for fluorine spectra. The assignment of resonance signals was based on the chemical shifts, intensities and the values of H,F or ^{13}C,F coupling constants. The analysis of proton spectra has not been performed and H,H coupling constants values were calculated as differences between the particular line positions in appropriate multiplets. Coupling constant values given for carbon spectra regard the F,C couplings. The sample for ^{19}F chemical shift measurements in water was prepared dissolving about 10 mg of **3** or **4** in 1 mL of water solution of bicarbonate (60 mg/ml; pH 8.5). Obtained solution was poured into 4 mm NMR tube, which was placed in 5 mm NMR tube containing deuterium oxide (lock reference) and a drop of 2,2,2-trifluoroethanol (chemical shift reference; δ = 0 ppm for central line of triplet).

4-Chloro-3-trifluoromethylbenzenesulfonic acid [2]: 1-Chloro-2-trifluoromethylbenzene (Aldrich, 10 g) was placed in the flask equipped with thermometer, dropping funnel and mechanical stirrer. Oleum (65%, 6.9 g) was added drop by drop to the cooled and vigorously stirred content of the flask keeping temperature below 0°C. After half of oleum was added, reacting mixture become a thick paste. It is

important to keep stirring and controlling the temperature until the end of oleum addition. The flask was left for 12 h in an ice-bath and then slowly warmed up to ambient temperature. Separated crystals of 4-chloro-3-trifluoromethylbenzenesulfonic acid were filtered off, washed twice with a small portion of cold water and dried in desiccator. Yield 1.5 g. Filtrate was poured in 50 ml of saturated NaCl solution. Precipitated sodium salt of sulfonic acid was collected, washed with aqueous NaCl solution and small volume of water and dried over P_2O_5 in vacuum. Yield 6.8 g. HRMS: $C_7H_4O_3F_3SCl$ requires *M* 259.9522, found 259.9526; ^{1}H NMR: 7.945 (d, 1H, H_2, $J_{H2,H6}$ = 1.4 Hz), 7.88 (dd, 1H, H_6, $J_{H6,H5}$ = 8.3 Hz), 7.68 (d, 1H, H_5); ^{13}C NMR: 147.64 (C_1), 131.67 (C_6), 131.27 (C_5, ^{4}J = 0.95 Hz), 130.97 (C_4, ^{3}J = 2.5 Hz), 126.14 (C_3, ^{2}J = 30.7 Hz), 124.80 (C_2, ^{3}J = 5.3 Hz), 122.77 (CF_3, ^{1}J = 271.6 Hz).

Sodium 4-chloro-3-nitro-5-trifluoromethylbenzenesulfonate, **2** [3]: The mixture of sodium 4-chloro-3-(trifluoromethyl)benzenesulfonate (2.0 g), nitric acid (d 1.54, 8.4 ml) and oleum (35%, 7.5 ml) was heated for 4 h at 90°C. Cooled mixture was then poured into 20 ml of saturated NaCl solution. Separated sodium 4-chloro-3-nitro-5-trifluoromethylbenzenesulfonate was filtered off, washed with brine, crystallized from a little of water and dried in vacuum to give 0.4 g of solid. It was then washed with chloroform to remove 1-chloro-2,4-dinitro-6-(trifluoromethyl)benzene. Remained solid was treated with 2 ml of warmed *N,N*-dimethylformamide. Obtained suspension was filtered through a pad of celite to separate inorganic salts. Solvent was removed from the filtrate under reduced pressure. The residue was washed with ether to remove remnant of DMF. Yield 0.26 g. HRMS: $C_7H_2O_5F_3SClNa$ requires $[M\text{-Na}]^-$ 303.9289, found 303.9269; ^{1}H NMR: 8.47 (dd, 1H, H_4 $J_{H4,H6}$ = 1.8 Hz), 8.18 (d, 1H, H_6); ^{13}C NMR: 149.61 (C_5), 148.88 (C_1), 128.59 (C_3, ^{2}J = 31.4 Hz), 127.35 (C_2 ^{3}J = 5.35 Hz), 125.87 (C_6), 123.33 (C_4, ^{3}J = 1.5 Hz), 121.89 (CF_3, ^{1}J = 272.2 Hz), (C_6); ^{19}F NMR: –74.16.

Synthesis of compounds **3**: The solution of aminoacid (2.1 mmol), sodium salt of compound **2** (1 mmol) and sodium bicarbonate (4 mmol) in water (1.5 ml) was placed in screw-capped ampoule and heated at 100°C for 18 h. The reaction mixture was then acidified with hydrochloric acid and extracted with ether. Ether layer was washed with brine, dried with $MgSO_4$ and evaporated to dryness at reduced pressure.

1-(2-Nitro-4-sulfo-6-trifluoromethylophenyl)pyrrolidin-2-carboxylic acid, **3a**. Yield: 89%; M.p. 161°C; R_f = 0.51; HRMS (EI): $C_{12}H_{10}N_2O_7F_3S$, requires $[M\text{-H}]^-$ 383.0176, found 383.0155; ^{1}H NMR (DMSO-d_6): 9.08 (vbs, 1H, OH), 8.14 (d, 1H, $H_{3'}$, $J_{H3',H5'}$ = 2.8 Hz), 8.05 (d, 1H, $H_{5'}$), 3.74 (dd, 1H, H_2, $J_{H2,H3}$ = 7.3 Hz, $J_{H2,H3B}$ = 6.7 Hz), 3.51 (m, 1H, H_{5A}), 3.46 (m, 1H, H_{5B}), 3.22 (m, 1H, H_{4A}), 2.101–1.97 (m, 3H, H_{3A} + H_{3B} + H_{4B}); ^{13}C NMR (DMSO-d_6): 172.29 (C_1), 149.68 ($C_{1'}$), 145.55 ($C_{4'}$), 139.96 ($C_{2'}$), 127.90 ($C_{5'}$, ^{3}J = 5.0 Hz), 126.02 ($C_{3'}$), 122.93 (CF_3, ^{1}J = 272.4 Hz), 118.03 ($C_{6'}$, ^{2}J = 29.6 Hz), 62.83 (C_2), 54.39 (C_5), 29.56 (C_3), 24.72 (C_4); ^{19}F NMR (DMSO-d_6): –58.90.

2-(2-Nitro-4-sulfo-6-trifluoromethylphenylamino)-3-methylpentanoic acid, **3b** (R = *sec*-butyl): Yield 79%; M.p. 138°C; ^{1}H NMR: 8.31 (d, 1H, $H_{3'}$, $J_{H3',H5'}$ = 2.8 Hz), 8.03 (d, 1H, $H_{5'}$), 6.94 (d, 1H, NH, $J_{NH,H2}$ = 6.9 Hz), 4.11 (dd, 1H, H_2, $J_{H2,H3}$ = 3.8 Hz), 2.82 (m, 1H, H_3), 1.91 (m, 2H, H_4), 1.77 (dd, 1H, H_{4A}, $J_{H4A,H4B}$ = –14.1 Hz, $J_{H4A,H3}$ = 3.8 Hz), 1.04 (dd, 1H, H_{4B}, $J_{H4B,H3}$ = 4.3 Hz), 0.97 (d, 3H, H_6, $J_{H6,H3}$ = 7.2 Hz), 0.92 (d, 3H, H_5, $J_{H5,H4}$ = 4.6 Hz); ^{13}C NMR: 172.98 (C_1), 141.76 ($C_{1'}$), 140.59 ($C_{4'}$), 139.26 ($C_{2'}$), 131.16 ($C_{5'}$, ^{3}J = 6.5 Hz), 128.08 ($C_{3'}$), 124.21 (CF_3, ^{1}J = 271.9 Hz), 119.55 ($C_{6'}$, ^{2}J = 30.4 Hz), 63.82 (C_2), 26.20 (C_3), 25.91 (C_4), 15.55 (C_5), 12.04 (C_6); ^{19}F NMR:–58.91.

2-(2-Nitro-4-sulfo-6-trifluoromethylphenylamino)-3-methylbutanoic acid, **3c** (R = $CH(CH_3)_2$): Yield 91%; M.p. 182°C; HRMS (EI): $C_{12}H_{12}N_2O_7F_3S$ requires $[M\text{-H}]^-$ 385.0333; found 385.0323; ^{1}H NMR: 8.22 (d, 1H, $H_{3'}$, $J_{H3',H5'}$ = 1.8 Hz), 7.971 (d, 1H, $H_{5'}$), 7.24 (d, 1H, NH, $J_{H2,NH}$ = 9.0 Hz), 3.76 (dd, 1H, H_2, $J_{H2,H3}$ = 3.4 Hz), 1.87 (dsept, 1H, H_3), 0.94 (d, 3H, $CH_{3(A)}$, $J_{CH3(A),H4}$ = 7.0 Hz), 0.79 (d, 3H, $CH_{3(B)}$, $J_{CH3(B),H4}$ = 6.8 Hz); ^{13}C NMR: 172.44 (C_1), 140.68 ($C_{1'}$), 139.00 ($C_{4'}$), 137.29 ($C_{2'}$), 130.08 ($C_{5'}$, ^{3}J = 6.1 Hz), 127.33 ($C_{3'}$), 124.65 (CF_3, ^{1}J = 272.0 Hz), 118.20 ($C_{6'}$, ^{2}J = 30.3 Hz), 63.89 (C_2, ^{5}J = 3.0 Hz), 30.15 (C_3), 18.06 ($CH_{3(A)}$), 17.44 ($CH_{3(B)}$); ^{19}F NMR: –60.55.

2-(2-Nitro-4-sulfo-6-trifluoromethylphenyloamino)acetic acid, **3d** (R = H): Yield 93%; M.p. 173°C; HRMS (EI): $C_9H_6N_2O_7F_3S$ requires $[M\text{-H}]^-$ 342.9875; found 342.9869; ^{1}H NMR: 8.08 (d, 1H, $H_{3'}$, $J_{H3',H5'}$ = 2.7 Hz), 7.86 (d, 1H, $H_{5'}$), 7.57 (d, 1H, NH, $J_{NH,H2}$ = 9.6 Hz), 3.05 (dq, 2H, H_2, $J_{H2,F}$ = 0.7 Hz);

^{13}C NMR: 173.47 (C_1), 138.94 ($C_{1'}$), 137.85 ($C_{4'}$), 135.46 ($C_{2'}$), 124.85 (CF_3, ^1J = 271.2 Hz), 128.10 ($C_{5'}$, ^3J = 5.6 Hz), 127.00 ($C_{3'}$), 116.78 ($C_{6'}$, ^2J = 30.0 Hz), 48.67 (C_2); ^{19}F NMR: –59.08.

2-(2-Nitro-4-sulfo-6-trifluoromethylphenylamino)propanoic acid, **3e** (R = CH_3): Yield 93%; M.p. 182°C; HRMS (EI): $C_{10}H_8N_2O_7F_3S$ requires $[M\text{-H}]^-$ 357.0023; found 356.9999; ^{1}H NMR: 9.04 (bs, 1H, COOH), 8.26 (d, 1H, $H_{3'}$, $J_{H3',H5'}$ = 2.8 Hz), 7.99 (d, 1H, $H_{5'}$), 7.57 (bs, 1H, NH, $J_{NH,H2}$ = 9.2 Hz), 3.99 (dq, 1H, H_2, $J_{H2,H3}$ = 6.8 Hz), 1.34 (d, 3H, H_3); ^{13}C NMR: 173.62 (C_1), 139.40 ($C_{1'}$), 136.98 ($C_{4'}$), 135.30 ($C_{2'}$), 128.84 ($C_{5'}$, ^3J = 5.6 Hz), 123.39 (CF_3, ^1J = 271.3 Hz), 117.52 ($C_{6'}$, ^2J = 29.2 Hz), 127.31 ($C_{3'}$), 54.86 (C_2, ^6J = 1.3 Hz), 18.09 (C_3); ^{19}F NMR: –60.19.

2-(2-Nitro-4-sulfo-6-trifluoromethylphenylamino)-3-hydroxypropanoic acid, **3f** (R = CH_2OH): Yield 87%; M.p. 183°C; HRMS (EI): $C_{10}H_8N_2O_8F_3S$ requires $[M\text{-H}]^-$ 372.9975; found 372.9969; ^{1}H NMR: 12.50 (bs, 1H, COOH), 8.28 (d, 1H, $H_{3'}$, $J_{H3',H5'}$ = 2.5 Hz), 8.00 (d, 1H, $H_{5'}$), 7.24 (d, 1H, NH, $J_{NH,H2}$ = 9.6 Hz), 4.04 (dt, 1H, H_2), 3.85 (dd, 1H, H_{3A}, $J_{H3A,H3B}$ = –10.8 Hz, $J_{H3A,H2}$ = 3.6 Hz), 3.69 (dd, 1H, H_{3B}, $J_{H3B,H2}$ = 2.8 Hz); ^{13}C NMR: 171.74 (C_1), 141.56 ($C_{1'}$, ^3J = 6.05 Hz), 139.84 ($C_{4'}$), 138.53 ($C_{2'}$), 130.72 ($C_{5'}$, ^3J = 5.9 Hz), 127.110 ($C_{3'}$), 123.52 (CF_3, ^1J = 272.0 Hz), 118.58 ($C_{6'}$, ^2J = 30.5 Hz), 61.41 (C_3), 59.39 (C_2, ^5J = 3.0 Hz); ^{19}F NMR: –58.81.

2-(2-Nitro-4-sulfo-6-trifluoromethylphenylamino)-3-(4-hydroxyphenyl)propanoic acid, **3h** (R = 4-hydroxybenzyl): Yield 93%; M.p. 187°C; HRMS (EI): $C_{16}H_{12}N_2O_8F_3S$ requires $[M\text{-H}]^-$ 449.0284; found 449.0282; ^{1}H NMR: 8.13 (d, 1H, $H_{3'}$, $J_{H3',H5'}$ = 2.8 Hz), 7.81 (d, 1H, $H_{5'}$), 7.57 (d, 1H, NH, $J_{NH,H2}$ = 8.5 Hz), 6.48–6.53 and 6.38–6.43 (4H, AA'BB' – Ph), 3.70 (d, 1H, H_2, $J_{H2,NH}$ = 5.7 Hz), 3.05 (d, 1H, H_{3A}, $J_{H3A,H3B}$ = –11.2 Hz), 3.97 (d, 1H, H_{3B}); ^{13}C NMR: 172.21 (C_1), 141.15 ($C_{1'}$), 139.73 ($C_{4'}$), 137.73 ($C_{2'}$), 129.91 ($C_{5'}$, ^3J = 5.7 Hz), 155.84, 130.16, 128.51, 128.36 (C_{Ar}), 127.03 ($C_{3'}$), 123.74 (CF_3, ^1J = 271.4 Hz), 117.72 ($C_{6'}$, ^2J = 30.6 Hz), 61.19 (C_2), 35.40 (C_3); ^{19}F NMR: –58.76.

2-(2-Nitro-4-sulfo-6-trifluoromethylphenylamino)-3-phenylpropanoic acid, **3g** (R = benzyl): Yield 84%; M.p. 167°C; ^{1}H NMR: 8.15 (d, 1H, $H_{3'}$, $J_{H3',H5'}$ = 2.6 Hz), 7.83 (d, 1H, $H_{5'}$), 7.55 (d, 1H, NH, $J_{NH,H2}$ = 6.8 Hz), 7.15 (m, 5H, H_{Ar}), 3.82 (m, 1H, H_2), 3.15 (d, 1H, H_{3A}, $J_{H3A,H3B}$ = –14.2 Hz), 3.10 (d, 1H, H_{3B}); ^{13}C NMR: 172.30 (C_1), 140.04 ($C_{1'}$), 137.10 ($C_{4'}$), 135.16 ($C_{2'}$), 137.00, 129.28, 127.61, 126.02 (C_{Ar}), 128.15 ($C_{5'}$, ^3J = 6.5 Hz), 127.41 ($C_{3'}$), 123.53 (CF_3, ^1J = 272.0 Hz), 117.23 ($C_{6'}$, ^2J = 29.6 Hz), 59.36 (C_2), 36.52 (C_3); ^{19}F NMR: –60.74.

Synthesis of 2-substituted 3-oxy-5-sulfo-7-trifluoromethyl-1H-benzimidazoles **4**. The solution of aminoacid (1 mmol), sodium salt of compound **2** (1 mmol) and sodium bicarbonate (6.2 mmol) in water (7 ml) was refluxed for 3 h. The mixture was acidified with hydrochloric acid and extracted with ether. Water layer was evaporated to dryness under reduced pressure and residue was treated with methanol (10 ml). Insoluble in methanol solid material was separated, dissolved in 5 ml of water and passed through the column packed with ion-exchange resin (Merck, Ionenaustascher I, art. 4765) using water as an eluent. After water removing with the use of rotary evaporator the residue was dried in vacuum.

4b (R = *sec*-butyl): Yield 59%; M.p. 138°C; ^{1}H NMR: 7.95 (d, 1H, H_4, $J_{H4,H6}$ = 2.6 Hz), 7.78 (d, 1H, H_6), 3.21 (m, 1H, C**H**CH_2, $Jc_{H,CH2}$ = 7.0 Hz), 1.83 (m, 2H, C$\mathbf{H}_2$), 1.34 (d, 3H, C$\mathbf{H}_3$), 1.11 (t, 3H, C$\mathbf{H}_3$); ^{13}C NMR: 157.39 (C_2), 143.03 (C_5), 131.99 (C_{3a}), 130.92 (C_{7a}), 123.54 (CF_3, ^1J = 272.1 Hz), 116.61 (C_7, ^2J = 32.4 Hz), 118.08 (C_6, ^3J = 4.6 Hz), 111.12 (C_4), 32.26 (CH), 27.26 (CH_2), 17.76 (CH_3), 11.81 (CH_3); ^{19}F NMR: –58.26.

4c (R = isopropyl): Yield 60%; M.p. 125°C; HRMS (EI): $C_{11}H_{11}N_2O_4F_3S$ requires $[M\text{-H}]^-$ 323.0322; found 323.0325; ^{1}H NMR: 7.93 (s, 1H, H_4), 7.77 (s, 1H, H_6), 3.45 (m, 1H, C**H**$(CH_3)_2$), 1.37 (d, 3H, $CH_{3(A)}$, $J_{CH3(A),CH}$ = 6.6 Hz), 1.28 (d, 3H, $CH_{3(B)}$, $J_{CH3(B),CH}$ = 6.4 Hz); ^{13}C NMR: 158.52 (C_2), 142.28 (C_5), 132.47 (C_{3a}), 132.77 (C_{7a}), 117.15 (C_7 ^2J = 27.0 Hz), 123.83 (CF_3, ^1J = 270.6 Hz), 117.26 (C_6, ^3J = 4.7 Hz), 110.69 (C_4), 25.56 (CH), 20.30 (CH_3); ^{19}F NMR: –58.99.

4d (R = H): Yield 60%; M.p. 131°C; HRMS (EI): $C_8H_5N_2O_4F_3S$ requires $[M\text{-H}]^-$ 280.9855, found 280.9849; ^{1}H NMR: 8.83 (s, 1H, H_2), 7.995 (s, 1H, H_4), 7.80 (s, 1H, H_6); ^{13}C NMR: 144.20 (C_2), 142.12 (C_5), 131.63 (C_{3a}), 133.63 (C_{7a}), 123.69 (CF_3, ^1J = 273.0 Hz), 118.04 (C_7, ^2J = 30.2 Hz), 117.85 (C_6, ^3J = 5.5 Hz), 111.31 (C_4); ^{19}F NMR: –58.90.

4e (R = CH_3): Yield 68%; M.p. 198° C; HRMS (EI): $C_9H_7N_2O_4F_3S$ requires $[M\text{-H}]^-$ 295.0005; found 294.9990; ^{1}H NMR: 7.94 (d, 1H, H_4 $J_{H4,H6}$ = 2.8 Hz), 7.79 (d, 1H, H_6), 2.64 (s, 3H, C$\mathbf{H}_3$); ^{13}C NMR:

151.25 (C_2), 142.87 (C_5), 131.83 (C_{3a}), 131.27 (C_{7a}), 123.59 (CF_3, ^1J = 271.0 Hz), 116.45 (C_7, ^2J = 31.2 Hz), 117.74 (C_6, ^3J = 4.6 Hz), 110.72 (C_4), 11.76 (CH_3); ^{19}F NMR: –58.91.

4f (R = CH_2OH): Yield 73%; M.p. 123°C; HRMS (EI): $C_9H_7N_2O_5F_3S$ requires $[M\text{-}H]^-$ 310.9970; found 310.9960; ^{1}H NMR: 7.97 (s, 1H, H_4), 7.80 (s, 1H, H_6), 4.77 (s, 2H, C**H**$_2$OH); ^{13}C NMR: 153.18 (C_2), 142.50 (C_5), 133.98 (C_{3a}), 132.40 (C_{7a}) 123.83 (CF_3, ^1J = 237.1 Hz), 117.30 (C_7, ^2J = 33.1 Hz), 117.01 (C_6, ^3J = 5.5 Hz), 110.60 (C_4), 59.44 (**C**H_2OH); ^{19}F NMR: –58.32.

4g (R = benzyl): Yield 53%; M.p. 173°C; ^{1}H NMR: 7.91 (d, 1H, H_4, $J_{H4,H6}$ = 2.5 Hz), 7.70 (d, 1H, H_6), 7.28 (m, 5H, H_{Ar}), 4.27 (s, 2H, C**H**$_2$); ^{13}C NMR: 152.63 (C_2), 141.84 (C_5), 132.44 (C_{3a}), 132.63 (C_{7a}), 124.00 (CF_3, ^1J = 271.5 Hz), 136.66, 128.75, 128.47, 126.53, (C_{Ar}), 116.70 (C_6, ^3J = 5.0 H), 116.80 (C_7, ^2J = 29.6 Hz), 110.56 (C_4), 32.45 (CH_2); ^{19}F NMR: –58.63.

4h (R = 4-hydroxybenzyl): Yield 57%; M.p. 153°C; HRMS (EI): $C_{15}H_{11}N_2O_5F_3S$ required $[M\text{-}H]^-$ 387.0293; found 387.0278; ^{1}H NMR: 7.89 (d, 1H, H_4, $J_{H4,H6}$ = 2.6 Hz), 7.50 (d, 1H, H_6), 7.19 (m, $H_{2,3,4,5,6}$), 4.14 (s, 2H, C**H**$_2$); ^{13}C NMR: 156.18 (C_2), 142.13 (C_5), 132.26 (C_{3a}), 132.63 (C_{7a}), 123.90 (CF_3, ^1J = 271.1 Hz), 153.30, 130.43, 129.74, 115.31 (C_{Ar}), 116.90 (C_7, ^2J = 31.0 Hz), 117.00 (C_6, ^3J = 5.0 Hz), 110.48 (C_4), 30.84 (CH_2); ^{19}F NMR: –58.32.

4i *3-(3-Oxy-5-sulfo-7-trifluoromethyl-1H-benzoimidazol-2-yl)-propionic acid* (R = $CH_2CH_2CO_2H$): ^{1}H NMR: 11.79 (bs, 1H, COOH), 7.91 (d, 1H, H_4, $J_{H4,H6}$ = 2.8 Hz), 7.70 (d, 1H, H_6), 3.10 (t, 2H, CH_2), 2.81 (t, 2H,C**H**$_2$$CO_2H$); ^{13}C NMR: 177.91 (COOH), 154.80 (C_2), 137.84 (C_5), 133.83 (C_{3a}), 134.73 (C_{7a}), 124.75 (CF_3, ^1J = 272.3 Hz), 114.69 (C_6, ^3J = 4.7 Hz), 115.62 (C_7, ^2J = 30.6 Hz), 111.55 (C_4), 35.63, 32.13 (2xCH_2); ^{19}F NMR: –58.69.

4j (R = $CH(OH)CH_3$): Yield 51 %; M.p. 103°C; ^{1}H NMR: 8.01 (d, 1H, H_4, $J_{H4,H6}$ = 2.6 Hz), 7.85 (d, 1H, H_6), 5.83 (bs, 1H, OH), 5.21 (q, 1H, H_1, $J_{CH,CH3}$ = 6.6 Hz), 1.63 (d, 3H, C**H**$_3$); ^{13}C NMR: 154.03 (C_2), 142.72 (C_5), 132.41 (C_{3a}), 123.80 (CF_3, ^1J = 272.3 Hz), 132.57 (C_{7a}), 123.80 (CF_3, ^1J = 272.3 Hz), 117.37 (C_7, ^2J = 30.6 Hz), 117.70 (C_6, ^3J = 4.7 Hz), 110.96 (C_4), 65.00 (CHOH), 21.27 (CH_3); ^{19}F NMR ; –58.69.

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