Acta Chemica Scandinavica, 1993: 47: 813–817
Printed in Belgium – all rights reserved

ACTA
CHEMICA SCANDINAVICA
ISSN 0904–213X

Synthesis and Characterization of *N*-Substituted Valines and their Phenyl- and Pentafluorophenyl-thiohydantoins

Per Rydberg,[a] Björn Lüning,[b] Carl Axel Wachtmeister[a] and Margareta Törnqvist[a,c,*]

[a] Department of Environmental Chemistry, Wallenberg Laboratory, [b] Department of Organic Chemistry and [c] Department of Radiobiology, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden

Rydberg, P., Lüning, B., Wachtmeister, C. A. and Törnqvist, M., 1993. Synthesis and Characterization of *N*-Substituted Valines and their Phenyl- and Pentafluorophenyl-thiohydantoins. – Acta Chem. Scand. 47: 813–817.

The phenylthiohydantoins of racemic *N*-methyl-, *N*-(2-hydroxyethyl)- and *N*-phenyl-valine as well as the pentafluorophenylthiohydantoins of these acids and of L-valine have been synthesized by reactions with phenyl isothiocyanate and pentafluorophenyl isothiocyanate, respectively. The synthesized thiohydantoins and the phenylthiohydantoin of L-valine were characterized by melting points, mass spectrometry, UV and ^{1}H NMR spectroscopy; the fluorinated thiohydantoins were also characterized by ^{13}C NMR spectroscopy. Among the *N*-substituted amino acids, racemic *N*-(2-hydroxyethyl)- and *N*-phenyl-valine, obtained from 2-bromoisovaleric acid with ethanolamine and aniline, respectively, were characterized by ^{1}H NMR spectroscopy and compared with respect to their pK_{a2}-values with L-valine and *N*-methyl-DL-valine.

Electrophilic compounds in living organisms may be identified by measurement of their products after reactions, or adduct formation, with nucleophilic sites in macromolecules.[1] Reactions with DNA may cause changes that ultimately lead to cancer. For identification and quantification of electrophiles *in vivo*, hemoglobin (Hb) offers certain advantages as a monitor molecule owing to its long and well-defined life span, as well as its availability in large amounts in blood samples.[1]

A sensitive and mild method, in principle a modification of the Edman protein-sequencing procedure,[2] has been developed for the determination of adducts to the *N*-termini (valines) in Hb.[3] In the conventional sequencing procedure the *N*-terminus is reacted with phenyl isothiocyanate (PITC) in a weakly alkaline aqueous medium, and, following acidification, the amino acid is eventually split off as a phenylthiohydantoin (PTH). The modified procedure (the '*N*-alkyl Edman method') is based on the observation that if the *N*-terminus is alkylated, the phenylthiohydantoin is split off without acidification.[4] High sensitivity in the determination of alkylated valines in Hb was achieved by utilizing the fluorinated Edman reagent, pentafluorophenyl isothiocyanate (PFPITC),[5] and by mass spectrometric analysis of the pentafluorophenylthiohydantoins (PFPTHs) of the substituted valines. The method has been used to monitor, in both humans and animals, a number of compounds such as epoxides and their precursor alkenes, methylating agents and aldehydes.[6] Several laboratories have adopted the method.[7]

Despite these applications, alkylated valines and their PTHs and PFPTHs have not been characterized, and the formation conditions of the latter from *N*-substituted valyl peptides have never been studied (cf. Ref. 8). As an initial step to gain this knowledge—a prerequisite for the definition of optimum conditions for analysis of different adducts—some substituted valines and their phenyl- and pentafluorophenyl-thiohydantoins have been synthesized and characterized.

Results and discussion

Structures of the compounds studied are given in Fig. 1. Throughout the work L-valine (**1**) and DL-forms of substituted valines (**2**, **3** and **4**) were used; since none of the substituents contain asymmetric carbons, no diastereomers of PTHs or PFPTHs were obtained.

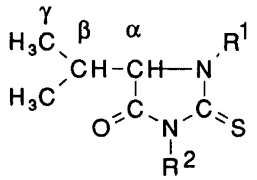
The reactions were followed by thin layer chromatography (TLC). The higher reactivity of PFPITC compared with PITC gives much faster coupling to the amino group.[9] The reaction to PFPTH was found to be complete within a few minutes after addition of PFPTC compared with about fifteen minutes for the formation of PTH from PITC. In the case of the alkylated valines no intermediate products were observed by TLC.

PhVal (**4**) shows a much lower pK'_{a2} than the other valines (**1**–**3**), which is analogous to the pK_a-difference for arylamines and alkylamines.[10] The PTH- and PFPITH-derivatives of PhVal (**8**) and (**12**) differ from those of the other compounds with regard to NMR shifts for the α and the β protons and UV spectra (λ_{max} longer, cf. Tables 1 and 3). It is worth noting that **4**, despite the low pK'_{a2} of the amino group readily gives rise to the ring-closed thiohydantoins upon treatment with the respective isothiocyanates.

With regard to applications of the *N*-alkyl Edman

* To whom correspondence should be addressed.

1 = L-valine (Val)
2 = *N*-methyl-DL-valine (MeVal)
3 = *N*-(2-hydroxyethyl)-DL-valine (HOEtVal)
4 = *N*-phenyl-DL-valine (PhVal)



5 $R^1 = H$, $R^2 = C_6H_5$ 5-isopropyl-3-phenyl-2-thiohydantoin (Val-PTH)
6 $R^1 = CH_3$, $R^2 = C_6H_5$ 5-isopropyl-1-methyl-3-phenyl-2-thiohydantoin (MeVal-PTH)
7 $R^1 = C_2H_4OH$, $R^2 = C_6H_5$ 1-hydroxyethyl-5-isopropyl-3-phenyl-2-thiohydantoin (HOEtVal-PTH)
8 $R^1 = R^2 = C_6H_5$ 5-isopropyl-1,3-diphenyl-2-thiohydantoin (PhVal-PTH)
9 $R^1 = H$, $R^2 = C_6F_5$ 5-isopropyl-3-pentafluorophenyl-2-thiohydantoin (Val-PFPTH)
10 $R^1 = CH_3$, $R^2 = C_6F_5$ 5-isopropyl-1-methyl-3-pentafluorophenyl-2-thiohydantoin (MeVal-PFPTH)
11 $R^1 = C_2H_4OH$, $R^2 = C_6F_5$ 1-hydroxyethyl-5-isopropyl-3-pentafluorophenyl-2-thiohydantoin (HOEtVal-PFPTH)
12 $R^1 = C_6H_5$, $R^2 = C_6F_5$ 5-isopropyl-3-pentafluorophenyl-1-phenyl-2-thiohydantoin (PhVal-PFPTH)

Fig. 1. Valines, phenylthiohydantoins (PTHs) and pentafluorophenylthiohydantoins (PFPTHs).

method for the determination of PFPTHs of *N*-substituted valines in Hb, mass spectrometric determination with negative chemical ionization (NCI-MS) offers the highest analytical sensitivity.[7] Analysis of the PFPTHs of Val, MeVal and HOEtVal by NCI-MS has been discussed earlier.[3,8] It has now been shown, as expected, that PhVal-PFPTH (**12**) is similar to MeVal-PFPTH (**10**) and differs from HOEtVal-PFPTH (**11**) with regard to the fragmentation pattern in NCI-MS (see Table 2). PFPTHs of MeVal and PhVal as well as of Val, lose a hydrogen to give the heaviest ion $[M-1]^-$, another important fragment being $[M-28]^-$ corresponding to loss of CO in the valyl residue. The ion $[M-103]^-$ [probably loss of $F+(CH_3)_2(CH)_2CO$ from the valyl residue] is a major fragment for MeVal-PFPTH (**10**) and PhVal-PFPTH (**12**), a minor fragment for HOEtVal-PFPTH (**11**) and is not observed from Val-PFPTH (**9**). HOEtVal-PFPTH (**11**), as well as other PFPTHs of *N*-(2-hydroxyalkyl)valines lose HF and give $[M-20]^-$ as the heaviest fragment (cf. Ref. 8). The fragment

Table 1. NMR data for PTHs and PFPTHs. ^1H NMR chemical shifts (δ), number of protons, multiplicity and coupling constants (*J*/Hz) of protons in compounds **5–12** dissolved in $CDCl_3$. The protons correspond to the letters given in Fig. 1.

	Valine spin system			
Compound	δ (*J*) CH(α) α–β	CH(β) β–α, β–γ,γ′	CH_3(γ,γ′) γ, γ′–β	R^1-spin system (cf. Fig. 1)
5	4.17 (2.9)	2.39, 2.40 (2.9, 7.0)	1.05, 1.14 (7.0)	7.90 (1 H, s)
6	4.02 (3.3)	2.45, 2.46 (3.3, 7.0)	1.03, 1.24 (7.0)	3.38 (3 H, s)
7	4.31 (3.3)	2.49, 2.50 (3.3, 7.0)	1.00, 1.27 (7.0)	α_1 3.64 (1 H, m), α_2 3.95 (1 H, m), β_1 4.05 (1 H, m), β_2 4.40 (1 H, m), OH 2.11 (1 H, t)
8	4.58 (3.3)	2.21, 2.22 (3.3, 7.0)	0.96, 1.21 (7.0)	arom. (5 H)
9	4.33 (3.7)	2.39, 2.40 (3.7, 7.0)	1.05, 1.14 (7.0)	8.60 (1 H, s)
10	4.13 (3.3)	2.45, 2.47 (3.3, 7.0)	1.02, 1.24 (7.0)	3.38 (3 H, s)
11	4.41 (3.7)	2.51, 2.52 (3.7, 7.0)	0.98, 1.26 (7.0)	α_1 3.64 (1 H, m), α_2 3.95 (1 H, m), β_1 4.05 (1 H, m), β_2 4.40 (1 H, m), OH 2.09 (1 H, t)
12	4.67 (3.3)	2.18, 2.22 (3.3, 7.0)	0.96, 1.20 (7.0)	arom. (5 H)

Table 2. Pertinent ions obtained in NCI–MS, PCI–MS and EI–MS.

Compound (No.)	*M*	*m/z* (NCI)[a]	*m/z* (PCI)[a]	*m/z* (EI)[a]
Val-PTH, (**5**)	234	233 (*M*−1, 100), 201 (*M*−33, 12)	235 (*M*+1, 100), 263 (*M*+29, 27), 275 (*M*+41, 8)	234 (*M*, 57), 205 (*M*−29, 12), 192 (*M*−42, 33), 136 (*M*−98, 38), 135 (*M*−99, 100), 91 (*M*−143, 28), 77 (*M*−157, 89)
MeVal-PTH, (**6**)	248	247 (*M*−1, 100), 232 (*M*−16, 7), 220 (*M*−28, 4), 205 (*M*−43, 8)	249 (*M*+1, 100), 277 (*M*+29, 27), 289 (*M*+41, 8)	248 (*M*, 60), 19 (*M*−29, 5), 206 (*M*−42, 100), 136 (*M*−112, 34), 135 (*M*−113, 29), 91 (*M*−157, 28), 77 (*M*−171, 74)
HOEtVal-PTH, (**7**)	278	277 (*M*−1, 35), 275 (*M*−3, 40), 233 (*M*−45, 100)	279 (*M*+1, 100), 307 (*M*+29, 33), 319 (*M*+41, 7)	278 (*M*, 17), 260 (*M*−18, 15), 218 (*M*−60, 44), 192 (*M*−86, 93), 173 (*M*−105, 25), 136 (*M*−142, 54), 135 (*M*−143, 66), 91 (*M*−187, 86), 77 (*M*−201, 100)
PhVal-PTH, (**8**)	310	309 (*M*−1, 100), 294 (*M*−16, 45), 265 (*M*−45, 12), 233 (*M*−77, 12), 190 (*M*−120, 12)	311 (*M*+1, 100), 339 (*M*+29, 37), 351 (*M*+41, 8)	310 (*M*, 26), 281 (*M*−29, 4), 268 (*M*−42, 11), 136 (*M*−177, 5), 135 (*M*−178, 7), 104 (*M*−206, 97), 91 (*M*−219, 25), 77 (*M*−233, 100)
Val-PFPTH, (**9**)	324	323 (*M*−1, 18), 296 (*M*−28, 100), 276 (*M*−48, 10), 225 (*M*−99, 30)	326 (*M*+2, 100), 353 (*M*+29, 26), 365 (*M*+41, 13)	324 (*M*, 84), 296 (*M*−28, 18), 282 (*M*−42, 71), 225 (*M*−99, 100)
MeVal-PFPTH, (**10**)	338	337 (*M*−1, 100), 319 (*M*−19, 8), 310 (*M*−28, 28), 235 (*M*−103, 50), 225 (*M*−113, 20)	340 (*M*+2, 100), 367 (*M*+29, 28), 379 (*M*+41, 12)	228 (*M*, 63), 310 (*M*−28, 5), 296 (*M*−42, 100), 277 (*M*−61, 28), 225 (*M*−113, 22)
HOEtVal-PFPTH, (**11**)	368	365 (*M*−3, 5), 347 (*M*−21, 100), 323 (*M*−45, 7), 318 (*M*−50, 10), 265 (*M*−103, 8), 225 (*M*−143, 5)	370 (*M*+2, 100), 397 (*M*+29, 26), 409 (*M*+41, 10)	368 (*M*, 18), 350 (*M*−18, 31), 325 (*M*−43, 12), 308 (*M*−60, 100), 282 (*M*−86, 77), 263 (*M*−105, 23), 225 (*M*−143, 55)
PhVal-PFPTH, (**12**)	400	399 (*M*−1, 100), 381 (*M*−19, 8), 372 (*M*−28, 13), 297 (*M*−103, 60), 225 (*M*−175, 15)	402 (*M*+2, 100), 429 (*M*+29, 26), 441 (*M*+41, 11)	400 (*M*, 100), 372 (*M*−28, 31), 358 (*M*−42, 63), 339 (*M*−61, 17), 317 (*M*−83, 17), 297 (*M*−103, 13), 225 (*M*−175, 26)

[a] Fragment, % relative intensity.

$[M-50]^-$ corresponds to additional loss of CH_2O from the HOEt-substituent and $[M-45]^-$ corresponds to loss of the whole HOEt-substituent. The fragment at *m/z* 225 corresponding to C_6F_5NCS is a major fragment for Val-PFPTH (**9**) and a minor, common fragment for all PFPTHs of *N*-substituted valines (cf. Ref. 11). The difference in fragmentation patterns between the phenylthiohydantions of HOEtVal and those of the other valines, and the similarities between the phenylthiohydantoins of the other valines, are also obvious in the MS-analysis of PTHs by NCI and electron impact ionization (EI), as well as in the analysis of PFPTHs by EI-MS.

Table 3. Yields and properties of PTHs and PFPTHs. Extinction coeff. (ϵ) and λ_{max} were measured in ethanol at a concentration of 10 mg l^{-1}.

	Val-		MeVal-		HOEtVal-		PhVal-	
Yield/property	PTH (**5**)	PFPTH (**9**)	PTH (**6**)	PFPTH (**10**)	PTH (**7**)	PFPTH (**11**)	PTH (**8**)	PFPTH (**12**)
Amino acid (mmol)	—	0.86	0.29	0.31	0.62	0.62	0.41	0.37
PITC or PFPITC (mmol)	—	1.7	1.2	0.64	1.9	1.2	1.2	0.74
Solvent (ml)	—	20	6	8	20	20	20	20
Yield (%)	—	89	82	85	82	90	80	85
Crystallized from	—	Heptane	Heptane	Heptane	Hexane–chloroform (4:1)	Ethanol–water (9:1)	Ethanol–water (1:1)	Ethanol–water (1:1)
$\epsilon/M^{-1}\,cm^{-1}$	16500[a]	16500	18500	16000	13500	20000	11000	14000
λ_{max}/nm	269	266	267	267	271	266	278	277
M.p./°C	206[b]	91–93.5	116–117	81–83	71–81[c]	90.5	154	83–84.5
R_f in TLC[d]	0.21	0.35	0.25	0.34	0.07	0.13	0.36	0.50
t_R/min in GLC[e]	10.52	9.10	10.39	8.54	12.34	10.43	13.59	11.49

[a] In agreement with values $\epsilon = 16500\ M^{-1}\,cm^{-1}$ and λ_{max} 269 nm given in Ref. 2. [b] Ref. 2 gives m.p. 206°C. [c] The wide m.p. interval is possibly due to the presence of two highly persistent impurities (ca. 1.5%) as revealed by GLC–FID. ^{13}C NMR confirmed that the compound was at least 95% pure. [d] Hexane–EtOAc 7:3 (v/v). [e] GLC conditions, see the Experimental.

Experimental

Chemicals. Pentafluorophenyl isothiocyanate (*purum*, PFPITC), phenyl isothiocyanate (*purum*, PITC) and 2-bromoisovaleric acid (98%) were obtained from Fluka, Buchs, Switzerland. L-Valine (**1**), *N*-methyl-DL-valine (**2**) and 5-isopropyl-3-phenyl-2-thiohydantoin (**5**) were obtained from Sigma, St. Louis, MO, USA. All other chemicals and solvents were of analytical grade.

Chromatography. All reactions were followed by TLC performed at room temperature on silica plates with a fluorescence indicator (Merck; DC-Fertigplatten, Kieselgel 60 F_{254}). TLC-R_f values were obtained by application of 10 μg aliquots of the compound to the plates and development with hexane–ethyl acetate 7:3 (v/v). The plates were exposed to ammonia in order to destroy the excess PFPITC or PITC and then the spots were visualized with UV light (254 nm). Preparative separations by liquid chromatography were performed on silica gel (Kieselgel 60, <0.063 mm; Merck, Darmstadt, FRG).

Instruments. Gas chromatographic (GLC) analyses were performed using a Shimadzu GLC-9AM gas chromatograph equipped with a DB-5 fused silica capillary column 30 m × 0.25 mm, 25 μm film thickness (J & W Scientific, Inc, CA, USA) and a flame ionization detector (FID). Temperature program: 80°C, 1 min; 10°C min^{-1} to 280°C, 4 min.

Gas chromatography–mass spectrometry (GLC–MS) was carried out on a Finnigan Model 4500 quadrupole instrument equipped with a 4500 Incos data system. The GLC separations were carried out with the column described above using helium as the carrier gas. The column temperature was kept at 100°C for 1 min, then increased to 320°C at a rate of 20°C min^{-1}. GLC–MS with negative chemical ionization (NCI) and positive chemical ionization (PCI) were performed with methane as the reagent gas, ion source pressure 0.45 Torr (60 Pa), ion source temperature 100°C, ionization energy 125 eV. GLC–MS with ionization by electron impact (EI) was performed with an ion source temperature of 140°C, electron energy 70 eV.

^1H and ^{13}C NMR spectra (270 MHz) were obtained in deuteriated chloroform ($CDCl_3$) at 25°C on a JEOL GSX 270 instrument using tetramethylsilane as an internal standard. Melting points were determined on a Kofler micro hot stage and are uncorrected. UV spectra were measured on a Hitachi U-3210 spectrophotometer. Elementary analyses were performed by Mikrokemi AB, Uppsala, Sweden.

Measurement of pH values was carried out on an Orion EA 920 pH-meter equipped with a Ross 8103 glass electrode and pK'_{a2}-values were determined by dissolving **1**, **2**, or **3** (0.25 mmol) in water (10 ml) and by dissolving **4** (0.25 mmol) as the hydrochloride in water (20 ml). **1**, **2**, **3** and **4** were titrated with 50.0 mM aqueous NaOH at 22°C. The determination was made graphically by plotting ΔV NaOH/Δ pH as a function of pH. The second dissociation constant (pK'_{a2}), was taken at 0.125 mmol NaOH (0.375 mmol for **4**) in the graph. The determination gave the following constants [pK'_{a2}]: **1** [9.7 (pK_{a2} 9.80, extrapolated from Ref. 12)], **2** [9.9], **3** [9.0] and **4** [4.4 ± 0.2].

Synthesis of N-*(2-hydroxyethyl)*-DL-*valine (***3**, *HOEtVal).* The synthesis was a modification of the method described by Calleman.[13] A mixture of 2-bromoisovaleric acid (7.50 g, 41.4 mmol), 2-aminoethanol (20 g, 330 mmol) and water (5 ml) was refluxed for 15 h and cooled to room temperature. Following precipitation with acetone (250 ml) and filtration, the product was dissolved in 1 M HCl (50 ml) and purified on a Dowex 50 ion-exchanger, washed with water (400 ml) and eluted with 2 M aqueous ammonia (300 ml). After evaporation to dryness, the residue was diluted with water (25 ml) and crystallized from acetone–ethanol [2 : 1 (v/v), 70 ml] to yield **3** 4.00 g (53.4%) as white crystals, m.p. 231–232°C. pK_{a2} 9.0. R_f-TLC 0.50 [propanol–water, 7:3 (v/v)]. ^1H NMR (D_2O, 25°C): δ 1.03, 1.08 [dd, 6 H, *J* 6.96 Hz, $CH_3(\gamma,\gamma')$], 2.27 [m, 1 H, *J* 6.96, 4.40 Hz, CH(β)], 3.22 (t, 2 H, *J* 5.13, 5.50 Hz, CH_2-1), 3.57 [d, 1 H, *J* 4.40, CH(α)], 3.87 (t, 2 H, *J* 5.13, 5.50 Hz, CH_2-2). Anal. $C_7H_{15}NO_3$: C, H, N.

Synthesis of N-*phenyl*-DL-*valine (***4**, *PhVal).* The synthesis was a modification of the method described by Bischoff.[14] 2-Bromoisovaleric acid (2.0 g, 11 mmol) was reacted with aniline (2.0 g, 22 mmol) in a Pyrex tube at 110–120°C (open flame) with vigorous shaking (decarboxylation occurs at 130°C). The reaction mixture solidified after 5–10 min as a yellowish-white mass, and was quickly chilled to room temperature and dissolved in ethanol (ca. 5 ml). The product precipitated after the addition of water (10 ml) and was crystallized from ethanol–water and dried protected from light *in vacuo** (1 mmHg, 60 h) to yield **4** 0.35 g (18%), m.p. 135–136°C (lit.[14] m.p. 137–138°C, lit.[15] m.p. 125°C). pK_{a2} 4.4 ± 0.2 (uncertainty of ±0.2 as **4** oiled out of solution during the titration). R_f-TLC 0.79 [propanol–water 7:3 (v/v)]. ^1H NMR (D_2O, 25°C): δ 1.05, 1.07 [dd, 6 H, *J* 6.98 Hz, $CH_3(\gamma,\gamma')$], 2.11 [m, 1 H, *J* 6.98, CH(β)], 3.72 [d, 1 H, *J* 6.98, CH(α)], 6.6–7.3 (m, 5 H, arom.). Found: C 67.4, H 7.85, N 7.40. Calc. for $C_{11}H_{15}NO_2$: C 68.40, H 7.82, N 7.25.

Structures for compounds **5**–**12** are given in Fig. 1. ^1H NMR chemical shifts and coupling constants are given in Table 1. Data from GLC–MS analysis are given in Table 2. Reaction conditions, yields, solvents for crystallization, ε, λ_{max}, m.p., R_f-TLC and retention times on GLC are collected in Table 3.

* To prevent the rapid light-induced destruction of **4** the crystallization must be carried out quickly and the substance should be stored protected from light in the refrigerator.

Synthesis of phenylthiohydantoins of N-*methylvaline (***6**, *MeVal-PTH),* N-*(2-hydroxyethyl)valine (***7**, *HOEtVal-PTH) and* N-*phenylvaline (***8**, *PhVal-PTH).* The respective *N*-substituted valines were dissolved in an aqueous mixture of 0.5 M $NaHCO_3$ and propanol (2:1) in pear-shaped flasks (cf. Refs. 3, 16). The solutions were placed with stirring in a water-bath at 45°C and PITC was added. The amounts of respective alkylvaline, solvent and reagent used are given in Table 3. Reactions were completed after 2 h. The reaction mixtures were extracted with heptane (10 ml) followed by centrifugation in Pyrex tubes equipped with Teflon-lined screw-caps. After four extractions the combined heptane–propanol phases were evaporated under nitrogen at 60°C. The resultant oils were purified on silica gel columns (2 × 25 cm) eluted with hexane–ethyl acetate [1 : 1 (v/v) for **6** and **7**, 7:3 for **8**]. The collected fractions were evaporated, dried *in vacuo* (1 mmHg, room temperature) and crystallized to yield **6**, **7** and **8** (purity determined on GLC–FID before recrystallisation was 100% for **6**, **8** and 98.5% for **7**).

6, Anal. $C_{13}H_{16}N_2OS$: C, H, N. **7**, Found: C 58.05, H 6.35, N 9.60; Calc. for $C_{14}H_{18}N_2O_2S$: C 60.41, H 6.52, N 10.06. **8**, Found: C 68.65, H 5.80, N 8.70; Calc. for $C_{18}H_{18}N_2OS$: C 69.65, H 5.84, N 9.02.

*Synthesis of the pentafluorophenylthiohydantoin of valine (***9**, *Val-PFPTH).* L-Valine (0.86 mmol) was dissolved in 0.5 M $NaHCO_3$ and propanol [2:1 (v/v), 20 ml]. PFPITC (1.7 mmol) was added and the mixture was kept 1 h at 45°C. Extraction with heptane (3 × 10 ml) was carried out to remove the excess of reagent and by-products. On addition of 6 M HCl (8 ml) to the aqueous phase the thiocarbamoylvaline precipitated; additional propanol (8 ml) was added to redissolve the precipitate (cf. Ref. 3). The solution was heated at 80°C with stirring for 0.5 h and the product (**9**), was extracted using heptane (3 × 40 ml). The heptane–propanol phase was evaporated under nitrogen at 60°C. The residue was purified on a silica gel column (2 × 25 cm) eluted with hexane–ethyl acetate [7:3 (v/v)] and dried *in vacuo* (1 mmHg, room temperature) to yield **9** (purity 100% GLC–FID). ^{13}C NMR ($CDCl_3$, 25°C): δ 16.03, 18.65 [CH_3 (γ,γ′)], 31.24 [CH(β)], 65.97 [CH(α)], 136–147 (C_6F_5), 171.3 (C=O), 181.6 (C=S). Anal. $C_{12}H_9F_5N_2OS$: C, H, N.

Synthesis of pentafluorophenylthiohydantoins of N-*methylvaline (***10**, *MeVal-PFPTH),* N-*(2-hydroxyethyl)valine (***11**, *HOEtVal-PFPTH) and* N-*phenylvaline (***12**, *PhVal-PFPTH).* The three reactions where run under the same conditions as described for the synthesis of **6–8** using pentafluorophenyl isothiocyanate (PFPITC) instead of PITC (cf. Refs. 3, 16). The amounts of *N*-alkylvalines, solvents and PFPTC used are given in Table 3. Extraction was performed as described for the synthesis of **6–8**. The resulting oils were purified on silica gel columns (2 × 25 cm), eluted with hexane–ethyl acetate [7:3 (v/v) for **10** and **12**, 1:1 for **11**]. Good separation of PFPTHs from by-products was obtained; the products (**10** white crystals, **11** and **12** oils) were dried *in vacuo* (1 mmHg, room temperature) and crystallized to yield **10**, **11**, and **12** as white crystals (purity determined on GLC–FID before recrystallization was 100% for **10**, **11** and **12**). **10** ^{13}C NMR ($CDCl_3$, 25°C): δ 15.94, 17.05 [CH_3 (γ,γ′)], 29.81 [CH(β)], 33.08 [R^1-(cf. Fig. 1)], 69.13 [CH(α)], 136–147 (C_6F_5), 170.1 (C=O), 179.8 (C=S). Anal. $C_{13}H_{11}F_5N_2OS$: C, H, N. **11** ^{13}C NMR ($CDCl_3$, 25°C): δ 15.38, 17.35 [CH_3 (γ,γ′)], 29.21 [CH(β)], 47.13, 60.56 [R^1-(cf. Fig. 1)], 68.75 [CH(α)], 136–146 (C_6F_5), 170.47 (C=O), 180.23 (C=S). Anal. $C_{14}H_{13}F_5N_2O_2S$: C, H, N. **12** ^{13}C NMR ($CDCl_3$, 25°C): δ 15.70, 17.24 [CH_3 (γ,γ′)], 29.97 [CH(β)], 70.02 [CH(α)], 126.7 (arom.), 128.7 (arom.), 129.6 (arom.), 136.6 (arom.), 136–147 (C_6F_5), 169.8 (C=O), 179.2 (C=S). Anal. $C_{18}H_{13}F_5N_2OS$: C, H, N.

Acknowledgments. We thank Lena Liedgren for measuring the NMR spectra. The work was supported financially by the Swedish Natural Science Research Council and the Swedish Environmental Protection Agency.

References

1. Ehrenberg, L. and Osterman-Golkar, S. *Teratog. Carcinog. Mutag. 1* (1980) 105.
2. Edman, P. and Henschen, A. In: Needleman, S. B., Eds., *Protein Sequence Determination*, 2nd ed., Springer-Verlag, Berlin and New York 1975, Chap. 8.
3. Törnqvist, M., Mowrer, J., Jensen, S. and Ehrenberg, L. *Anal. Biochem. 154* (1986) 255.
4. Jensen, S., Törnqvist, M. and Ehrenberg, L. In: de Serres, F. J. and Pero, R. W., Eds., *Individual Susceptibility to Genotoxic Agents in the Human Population*, Plenum Press, New York 1984, p. 315.
5. Lequin, R. M. and Niall, H. D. *Biochim. Biophys. Acta 257* (1972) 76.
6. Törnqvist, M. In: Garner, R. C., Farmer, P. B., Steel, G. T. and Wright, A. S., Eds., *Human Carcinogen Exposure: Biomonitoring and Risk Assessment*, Oxford University Press, Oxford and New York 1991, Chap. 37, p. 411.
7. Törnqvist, M., Magnusson, A.-L., Farmer, P. B., Tang, Y.-S., Jeffrey, A. M., Wazneh, L., Beulink, G. D. T., van der Waal, H. and van Sittert, N. J. *Anal. Biochem. 203* (1992) 357.
8. Törnqvist, M. *Ph. D. Thesis*, University of Stockholm, Stockholm 1989.
9. Schneider, M. and Tschesche, H. *Hoppe-Seyler's Z. Physiol. Chem. 357* (1976) 1339.
10. Noller, C. R. *Chemistry of Organic Compounds*, 3rd ed., Saunders, Philadelphia and London 1965, p. 986.
11. Fairwell, T. and Brewer, H. B. *Anal. Biochem. 107* (1980) 140.
12. Edsall, J. T. In: Cohn, E. J. and Edsall, J. T., Eds., *Proteins, Amino Acids and Peptides as Ions and Dipolar Ions*, Reinhold, New York 1943, Chap. 4.
13. Calleman, C. J. *Ph. D. Thesis*, University of Stockholm, Stockholm 1984.
14. Bischoff, C. A. *Ber. Dtsch. Chem. Ges. 30* (1897) 2308.
15. Gal, E. M. *J. Am. Chem. Soc. 71* (1949) 2253.
16. Mowrer, J., Törnqvist, M., Jensen, S. and Ehrenberg, L. *Toxicol. Environ. Chem. 11* (1986) 215.

Received October 9, 1992.