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Synthesis of Pentafluorophenylthiohydantoin Derivatives of Adducts Formed with *dl*-Valine by Acrylonitrile and Cyanoethylene Oxide

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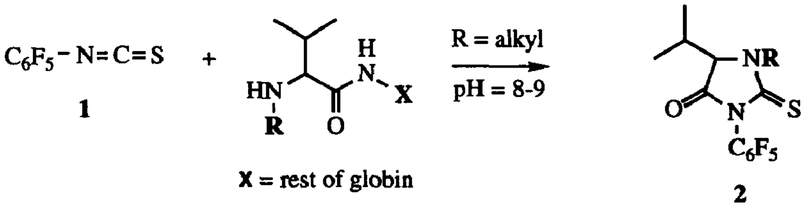
Abstract: Pentafluorophenylthiohydantoin (PFPTH) derivatives of adducts formed with *dl*-valine by both acrylonitrile and its putative metabolite, cyanoethylene oxide have been synthesised, the latter *via* conjugate addition of *dl*-valine to phenyl vinyl sulfoxide and subsequent Pummerer rearrangement and hydrolysis.

The monitoring of human exposure to carcinogens is an important area of research because it can provide useful information with respect to cancer risk assessment.[1] Many carcinogens are electrophilic, or are metabolised to electrophilic species which react to form covalent adducts with nucleophilic sites on DNA and proteins.[2] Although DNA adducts are thought to be critical for the initiation of the carcinogenic process[3,4] the monitoring of protein adducts offers a relevant measure of extent of exposure to a carcinogen over an extended period.[5]

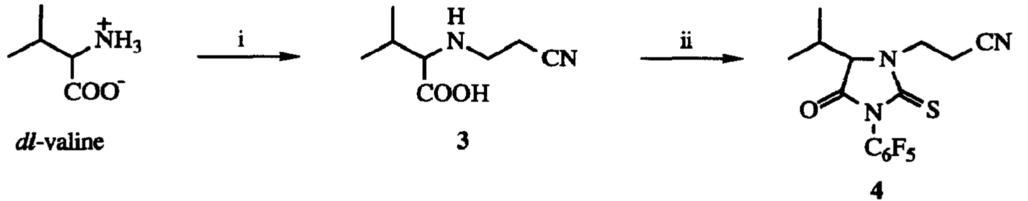
Alkylated *N*-terminal valine in haemoglobin is a major adduct formed following the reaction of haemoglobin with electrophilic carcinogens *in vivo* although the extent of reaction is dependent on the nature of the electrophile.[1] The alkylated *N*-terminal valine can be selectively cleaved using a modified Edman degradation procedure[6] and involves coupling the alkylated valine with pentafluorophenylisothiocyanate **1** under mildly basic conditions to produce a pentafluorophenylthiohydantoin (PFPTH) derivative **2** (Scheme 1) which can be analysed by GC/MS.



Scheme 1

This methodology has been successfully applied to the monitoring of human exposure to ethylene oxide[6-9] propylene oxide[6,9] and acrylamide[10] in work environments whilst adducts with acrylonitrile and cyanoethylene oxide are under investigation.[11] Cyanoethylene oxide is produced *via* oxidation of acrylonitrile *in vivo* by cytochrome P450 and has been proposed as the putative toxicant responsible for the carcinogenic effects of acrylonitrile.[12] Work has been initiated on developing an assay for both acrylonitrile and cyanoethylene oxide exposure and the synthesis and characterisation of authentic samples of these adducts following modified Edman degradation is reported in this letter.

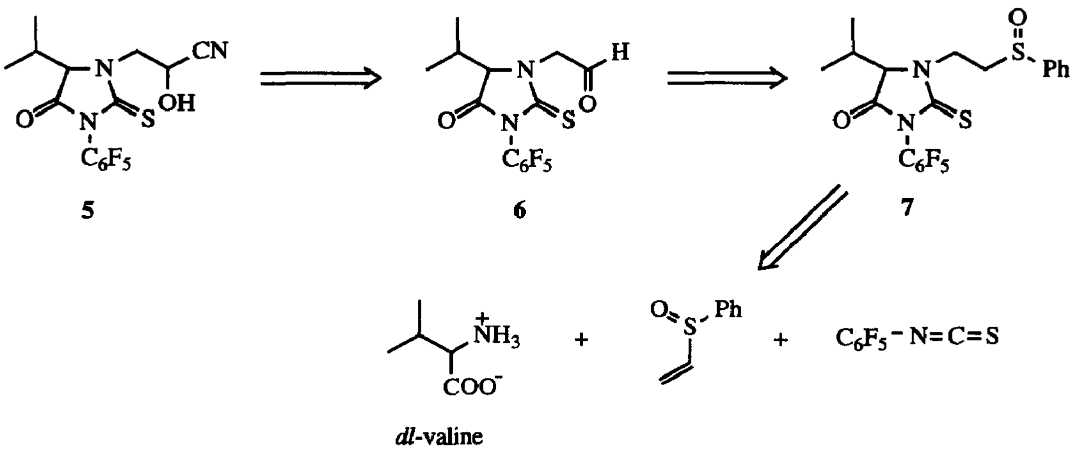
Cyanoethylation of *dl*-valine with acrylonitrile using an equivalent of base[13] afforded *N*-cyanoethylvaline **3** in 64% yield. Cyclisation of the alkylated valine with pentafluorophenylisothiocyanate under mildly basic conditions produced the PFPTH derivative **4** (56%) (Scheme 2).



Scheme 2

Reagents: [i], $CH_2{=}CHCN$ (1.1eq), aq.NaOH (1eq), 10-25°, 24h, then H^+(1eq); [ii], C_6F_5NCS (1.1eq), $KHCO_3$, 2-propanol, pH 8.5, 45°, 2h.

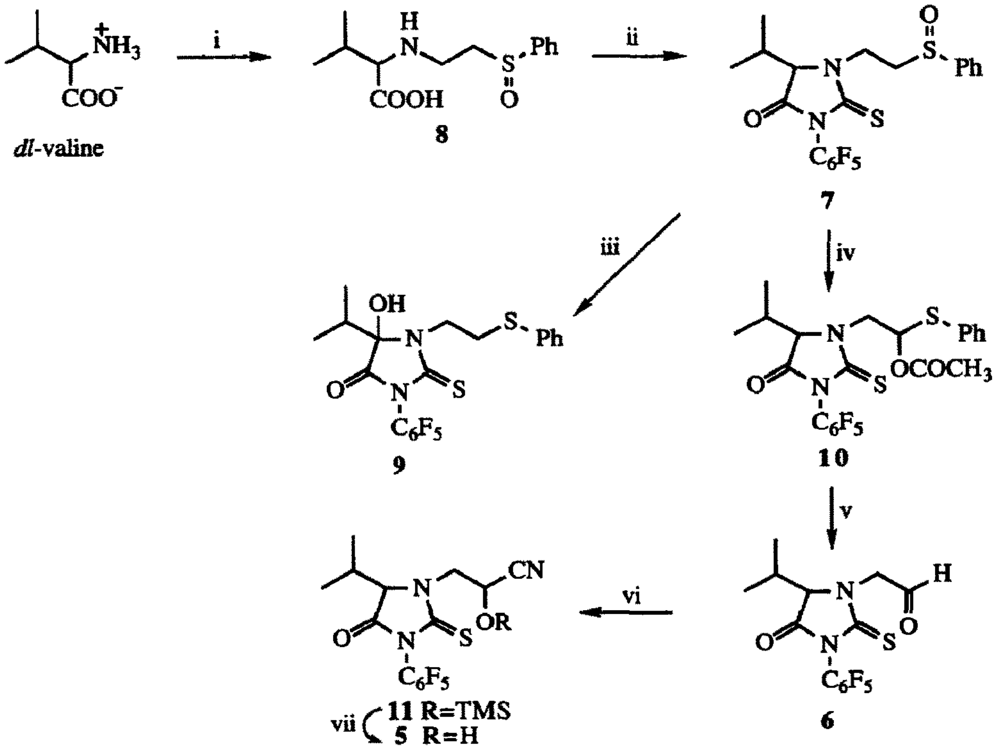
It was envisaged that the analogous reaction involving conjugate addition of *dl*-valine to cyanoethylene oxide would not be favourable under the reaction conditions employed and so an alternative was sought. Retrosynthetic analysis of the PFPTH-cyanoethylene oxide adduct **5** suggested the aldehyde **6** as a key intermediate following functional group interconversion of the sulfoxide **7** (Scheme 3).



Scheme 3

Conjugate addition of *dl*-valine to phenyl vinyl sulfoxide afforded the alkylated valine **8** (56%) which was coupled with pentafluorophenylisothiocyanate in pyridine to yield the PFPTH derivative **7** (46%) after flash chromatography (silica; 66% ether/light petroleum) and as a 1:1 mixture of diastereomers (Scheme 4). Conversion of aryl sulfoxides directly into aldehydes using trifluoroacetic anhydride has been reported,[14] but, in this study, formation of the remote hydroxylated product **9** (87%) was observed, the identity of which was confirmed by ^{13}C n.m.r. and mass spectroscopy.[15] Pummerer rearrangement of sulfoxide **7** with acetic anhydride/sodium acetate[16] afforded the α-acetoxysulfide **10** (97%) (1:1 diasteromeric mixture) which resisted conventional hydrolysis conditions.[14,17] A mercuric chloride/sulfuric acid mediated hydrolysis eventually yielded the aldehyde **6** (30%) following radial chromatography (silica; 20% ether/light petroleum).

A sample of the *O*-TMS-cyanohydrin **11** was required for GC/MS analysis in a related study. Cyanosilylation of the aldehyde **6**[18] with cyanotrimethylsilane in the presence of catalytic zinc iodide (0.02 eq.) gave the *O*-TMS-cyanohydrin **11** (30%) (1:1 diastereomeric mixture after purification by HPLC) which proved to be stable in anhydrous aprotic solvents. Hydrolysis of **11** with 2M HCl[19] yielded the required cyanohydrin **5** (98%) (1:1 mixture of diastereomers). Attempted GC/MS[20] analysis of **5** was not successful with the cyanohydrin reverting completely to the aldehyde **6**.



Scheme 4

Reagents: [i], $CH_2=CHSOPh$ (1.1eq), aq. NaOH (1eq), 10-25°, 24h, then H^+(1eq); [ii], C_6F_5NCS (1.1eq), pyridine, 45°, 3h; [iii], $(CF_3CO)_2O$, 2,6-lutidine, 0°, 15min then $NaHCO_3/H_2O$, 2h; [iv], $(CH_3CO)_2O$, CH_3COONa (eq wt. to 7), reflux, 5h; [v], $HgCl_2$ (1.2eq), 20% aq. THF, 2 drops conc. H_2SO_4, 20-65°, 24h; [vi], TMSCN (1.1eq), ZnI_2 (0.02eq), anhydrous DCM, 20°, 22h; [vii], 2M HCl, THF, 30°, 2h.

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15. *Spectral data for selected compounds:*

4: ^{1}H NMR (500MHz, CDCl$_3$): 1.01, d, *J* 7.0 Hz, C*H*$_3$; 1.30, d, *J* 7.0 Hz, C*H*$_3$; 2.48-2.55, m, C*H*(CH$_3$)$_2$; 2.82, dt, *J* 17.2, 5.5 Hz, CH(*H*)CN; 3.12, ddd, *J* 16.6, 8.3, 6.5 Hz, C*H*(H)CN; 3.83, ddd, *J* 14.2, 8.5, 6.2 Hz, NCH(*H*); 4.37, dt, *J* 14.4, 5.6 Hz, NC*H*(H); 4.41, d, *J* 3.2 Hz, ring proton. ^{13}C NMR (75MHz, CDCl$_3$): 15.4 *C*H$_3$, 15.8 *C*H$_2$CN, 17.2 *C*H$_3$, 29.4 *C*H(CH$_3$)$_2$, 41.2 N*C*H$_2$, 68.0 *C*HCO, 117.1 *C*N, 169.1 *C*O, 179.0 *C*S. GC/MS(EI), t$_R$ 7.49min.: 377(M, 78), 335 (100), 282(81), 263(24), 225(33), 194(16), 167(10), 117(10), 83(44).

5: ^{1}H NMR (500MHz, CDCl$_3$): 0.96, d, *J* 7.0 Hz, C*H*$_3$ for one diast.; 0.98, d, *J* 6.9 Hz, C*H*$_3$ for other diast.; 1.26, d, *J* 6.9 Hz, C*H*$_3$ for one diast.; 1.28, d, *J* 7.0 Hz, C*H*$_3$ for other diast.; 1.95, bs, O*H*; 2.48-2.56, m, C*H*(CH$_3$)$_2$; 3.74, dd, *J* 14.7, 9.4 Hz, NCH(*H*) for one diast.; 3.84, dd, *J* 14.4, 5.4 Hz, NCH(*H*) for other diast.; 4.43, d, *J* 3.4 Hz, ring proton for one diast.; 4.50, d, *J* 3.5 Hz, ring proton for other diast.; 4.56, dd, *J* 14.5, 6.8 Hz, NC*H*(H) for one diast.; 4.64, dd, *J* 14.4, 3.0 Hz, NC*H*(H) for other diast.; 5.10, m, C*H*(OH)CN for both diast. ^{13}C NMR (75MHz, CDCl$_3$): 15.1, 15.3 *C*H$_3$ for both diast.; 17.0, 7.2 *C*H$_3$ for both diast.; 29.0, 29.1 *C*H(CH$_3$)$_2$ for both diast.; 47.6, 48.1 *C*H$_2$; 58.6, 58.9 *C*H(OH)CN for both diast.; 68.2, 69.0 *C*HCO for both diast.; 117.2, 117.6 *C*N for both diast.; 169.4, 169.6 *C*O for both diast.; 180.4 *C*S for both diast. MS(FAB+): 394(M+H, 74), 367(36), 226(25).

6: ^{1}H NMR (500MHz, CDCl$_3$): 1.05, d, *J* 6.7 Hz, C*H*$_3$; 1.26, d, *J* 6.7 Hz, C*H*$_3$; 2.26-2.35, m, C*H*(CH$_3$)$_2$; 4.38, d, *J* 18.8 Hz, CH(*H*)CO; 4.40, d, *J* 2.7 Hz, ring proton; 5.13, d, *J* 18.8 Hz, C*H*(H)CO; 9.72, s, CH*O*. ^{13}C NMR (75MHz, CDCl$_3$): 15.7 *C*H$_3$, 17.1 *C*H$_3$, 29.6 *C*H(CH$_3$)$_2$, 54.4 *C*H$_2$, 67.9 *C*HCO, 193.6 H*C*O. GC/MS(EI), t$_R$ 7.18min.: 366(M, <1), 338 (80), 296(100), 277(30), 225(27), 193(10), 167(8), 84(48), 55(47).

7: ^{1}H NMR (500MHz, CDCl$_3$): 0.96, two overlapping d, *J* 6.8 Hz, C*H*$_3$ for both diast.; 1.22 and 1.28, d, *J* 7.0 Hz, C*H*$_3$ for both diast.; 2.40-2.50, m, C*H*(CH$_3$)$_2$ for both diast.; 3.02, dt *J* 13.6, 5.5 Hz, CH(*H*)SOPh for one diast.; 3.35-3.47, m, C*H*(H)SOPh for both diast.; 3.63, dt, *J* 13.8, 7.3 Hz, CH(*H*)SOPh for other diast.; 3.86-3.94, m, NCH(*H*) for both diast.; 3.98, d, *J* 3.4 Hz, ring proton for one diast.; 4.33-4.39, m, NC*H*(H) for both diast.; 4.48, d, *J* 3.5 Hz, ring proton for other diast. ^{13}C NMR (75MHz, CDCl$_3$): 15.2 *C*H$_3$ for both diast.; 17.0, 17.1 *C*H$_3$ for both diast.; 29.2 *C*H(CH$_3$)$_2$ for both diast.; 38.3, 38.5 *C*H$_2$SOPh for both diast.; 51.6, 52.7 N*C*H$_2$ for both diast.; 66.0, 66.1 *C*HCO for both diast.; 123.6, 129.3, 129.4, 131.1, 131.2 Ar*C*H; 142.0 Ar*C*; 169.5, 169.6 *C*O for both diast.; 179.7 *C*S for both diast. MS(FAB+): 477 (M+H, 12), 351(45), 323(33), 137(23).

9: ^{1}H NMR (500MHz, CDCl$_3$): 0.91, d, *J* 7.1 Hz, C*H*$_3$; 1.18, d, *J* 6.9 Hz, C*H*$_3$; 2.23-2.45, m, C*H*(CH$_3$)$_2$; 3.32, ddd, *J* 13.9, 9.5, 4.8 Hz, C*H*(H)SPh; 3.47, ddd, *J* 13.8, 9.6, 6.3 Hz, CH(*H*)SPh; 3.65, ddd, *J* 14.2, 9.5, 6.2 Hz, NC*H*(H); 4.17, ddd, *J* 14.3, 9.6, 4.9 Hz, NCH(*H*); 7.23, d, *J* 7.4 Hz, Ar(*H*4); 7.30, t, *J* 7.5 Hz, Ar(*H*3, *H*5); 7.44, d, *J* 7.8 Hz, Ar(*H*2, *H*6). ^{13}C NMR (75MHz, CDCl$_3$): 15.0 *C*H$_3$, 15.7 *C*H$_3$; 30.6 *C*H$_2$SPh; 34.4 *C*H(CH$_3$)$_2$; 43.0 N*C*H$_2$; 91.1 *C*(OH); 126.8, 129.1, 129.6 Ar*C*H; 134.1 Ar*C*. MS(EI): 476(M, 43), 367(17), 323(5), 269(15), 225(8), 136(100), 109(27), 91(17).

10: ^{1}H NMR (500MHz, CDCl$_3$): 0.92, two overlapping d, *J* 6.9 Hz, C*H*$_3$ for both diast.; 1.22, two overlapping d, *J* 7.3 Hz, C*H*$_3$ for both diast.; 2.08 and 2.10, s, C*H*$_3$CO for both diast.; 2.28-2.40, m, C*H*(CH$_3$)$_2$ for both diast.; 3.55, dd *J* 14.7, 7.8 Hz, NCH(*H*) for one diast.; 3.66, dd, *J* 14.7, 4.6 Hz, NCH(*H*) for other diast.; 4.21, d, *J* 3.4 Hz, ring proton for one diast 4.42, d, *J* 3.5 Hz, ring proton for other diast.; 4.89, two overlapping dd, *J* 14.7, 7.8 Hz, NC*H*(H) for both diast.; 6.43, dd, *J* 8.0, 4.6 Hz, C*H*(OAc) for one diast.; 6.48, dd, *J* 7.8, 5.0 Hz, C*H*(OAc) for other diast; 7.32-7.40, m, Ar*H*.. ^{13}C NMR (75MHz, CDCl$_3$): 15.6 *C*H$_3$ for both diast.; 17.2 *C*H$_3$ for both diast.; 28.9, 29.1 *C*H(CH$_3$)$_2$ for both diast; 47.4 N*C*H$_2$ for both diast.; 67.0, 68.1 *C*HCO for both diast; 76.3, 76.8 *C*H(OAc) for both diast.; 128.9 Ar*C*, 129.3, 133.5, 134.1 Ar*C*H. GC/MS(EI), t$_R$ 10.44min.: 518(M, 37), 409(100), 367(85), 349(95), 321(62), 296(33), 225(26), 152(85), 136(35), 110(82).

11: ^{1}H NMR (500MHz, CDCl$_3$): 0.25 and 0.28, s, Si(C*H*$_3$)$_3$ for both diast.; 0.96, d, *J* 6.9 Hz, C*H*$_3$ for one diast.; 0.98, d, *J* 6.9 Hz, C*H*$_3$ for other diast.; 1.26 and 1.27, two d, *J* 7.0 Hz, C*H*$_3$ for both diast.; 2.48-2.58, m, C*H*(CH$_3$)$_2$ for both diast. 3.63, dd *J* 14.2, 9.7 Hz, NCH(*H*) for one diast.; 3.71, dd *J* 14.1, 6.9 Hz, NCH(*H*) for other diast.; 4.33, d, *J* 3.5 Hz, ring proton for one diast.; 4.49, d, *J* 3.4 Hz, ring proton for other diast.; 4.52, dd, *J* 14.1, 8.3 Hz, NC*H*(H) for one diast.; 4.56, dd, *J* 14.2, 3.4 Hz, NC*H*(H) for other diast.; 5.12, dd, *J* 6.6, 6.2 Hz, C*H*(OTMS) for one diast.; 5.21, dd, *J* 9.5, 3.4 Hz, C*H*(OTMS) for other diast. ^{13}C NMR (75MHz, CDCl$_3$): -0.69, -0.47 Si(*C*H$_3$)$_3$ for both diast.; 15.4, 15.5 *C*H$_3$ for both diast.; 17.2, 17.3 *C*H$_3$ for both diast.; 29.1 *C*H(CH$_3$)$_2$ for both diast; 48.5, 48.9 N*C*H$_2$ for both diast.; 58.1, 58.3 *C*H(OTMS) for both diast.; 66.2, 69.5 *C*HCO for both diast; 117.4, 118.4 *C*N for both diast.; 169.5, 169.7 *C*O for both diast. GC/MS(EI), t$_R$ 7.54 and 8.01min.: 465(M, 81), 450(48), 337(70), 296(22), 282(100), 225(15), 84(70), 73(57).

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20. GC conditions: Hewlett Packard HP5890R GC equipped with a 25m x 0.32mm SE52 fused silica capillary column. Injections were made with an initial column temp. of 80° for 1min. then at 30°/min to 280°.

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