JOC*Note*

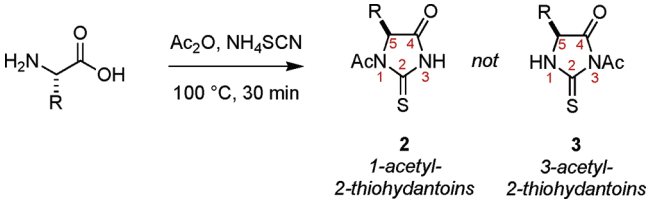
On Formation of Thiohydantoins from Amino Acids under Acylation Conditions

Samuel Reyes and Kevin Burgess*

Texas A & M University, Chemistry Department, P.O. Box 30012, College Station, Texas 77842

burgess@tamu.edu

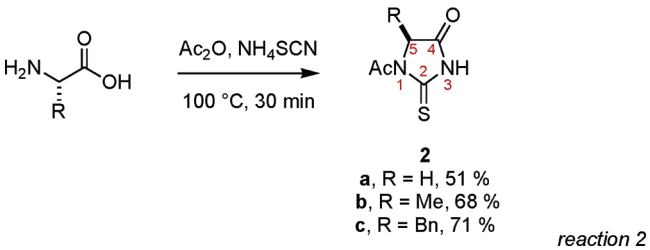
Received December 14, 2005



Reactions of glycine, alanine, and phenylalanine with acetic anhydride and ammonium thiocyanate give the 1-acetyl-2-thiohydantoins **2a**−**c**. These results appear to contradict prior literature reports pertaining to this reaction.

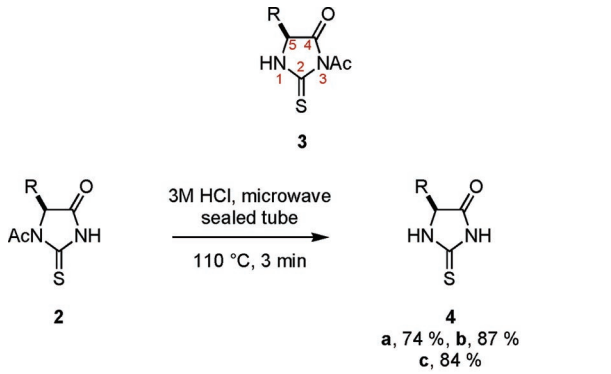
Reactions of aryl isothiocyanates with (*N*-termini-unmasked) peptides represent the Edman degradation for peptide sequencing.[1] The ultimate reaction products of these transformations, 2-thiohydantoins, are also conveniently formed from amino acid esters and aryl isothiocyanates, as shown in reaction 1.[2] This synthetic approach is useful for the preparation of heterocycles from peptides and, further, has potential application in combinatorial chemistry.[3] Hence, researchers in the field may also consider similar reactions applied to *unprotected* amino acids under acylating conditions (reaction 2). We were interested in exploring this chemistry, but found the literature pertaining to these reactions to be confusing, ambiguous, and, in isolated minor cases, incorrect. This paper is intended to clarify the situation.

reaction 1



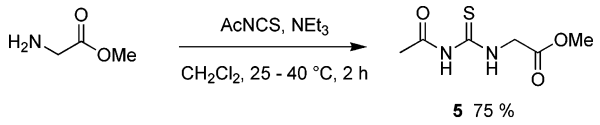
reaction 2

In addition to **2a**−**c**, other plausible products from reaction 2 include the isomeric 3-acylthiohydantoins **3**. Spectroscopic (NMR) evidence provides support for the results that reaction 2 affords products **2**, shown above, and *not* structures **3**. First, the proton chemical shift of the N[3]*H* in DMSO-d_6 appears in the region of 12.6 ppm, markedly downfield with respect to the chemical shifts typically associated with cyclic thioamides (ca. 9.15 ppm).[4] Second, no 2D COSY C[5]*H*-to-N[1]*H* correlation cross-peaks were observed for **2a**−**c**, whereas a corresponding cross-peak was observed for compound **1** as well as in the COSY spectrum of products **4** (reaction 3). Finally, a new N*H* signal for the deacylated products gave chemical shifts consistent with cyclic thioamides.



reaction 3

We attempted to prepare compound **3a** via reaction 4, but instead obtained the acyclic thioamide product **5**. This material when treated with a suitable base (K_2CO_3, MeOH, 25 °C, 1 h) failed to cyclize to **3a**. In any event, it is to be expected that compounds **3** would tend to lose an acyl group even to weak nucleophiles, i.e., it would not be particularly stable in the presence of nucleophiles.



reaction 4

Confusion about the products of reaction 2 arose because the numbering system for hydantoins and thiohydantoins changed prior to and after 1907.[5] Figure 1 illustrates the difference in numbering before and after 1907. Both Chemical Abstracts and IUPAC[5,6] have adopted the nomenclature appearing for these compounds *after* 1907.

In 1911, Johnson et al. published a preparation for compound **2a** via the conditions shown in reaction 2,[7] and subsequently others in the series including **2b** and **2c**.[8,9] They referred to them

(1) Edman, P.; Begg, G. *Eur. J. Biochem.* **1967**, *1*, 80−91.
(2) LeTiran, A.; Stables, J. P.; Kohn, H. *Bioorg. Med. Chem.* **2001**, *9*, 2693−2708.
(3) Evindar, G.; Batey, R. A. *Org. Lett.* **2003**, *5*, 1201−4.
(4) Smith, D. C.; Lee, S. W.; Fuchs, P. L. *J. Org. Chem.* **1994**, *59*, 348−54.
(5) Ware, E. *Chem. Rev.* **1950**, *46*, 403−70.
(6) *A Guide to IUPAC Nomenclature of Organic Chemistry, Recommendations 1993*; Blackwell Scientific Publications: Oxford, 1993.
(7) Johnson, T. B.; Nicolet, B. H. *J. Am. Chem. Soc.* **1911**, *33*, 1973−8.
(8) Johnson, T. B. *Am. Chem. J.* **1913**, *49*, 197−204.

10.1021/jo052576i CCC: $33.50 © 2006 American Chemical Society
Published on Web 02/14/2006

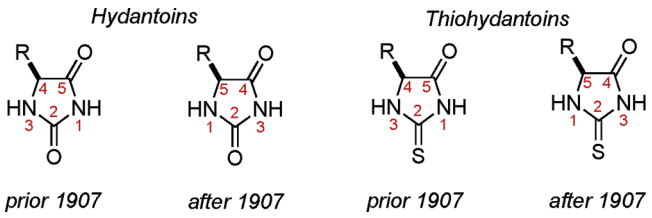


FIGURE 1. Numbering system on hydantoins and thiohydantoins before and after 1907.

as *3-acyl-2-thiohydantoins*, following the accepted nomenclature *before* 1907. Following Johnson's studies, another group, more than 65 years later, reported the transformation of glycine into "3-acyl-2-thiohydantoin" but did not draw a structure or give spectral data.[10] The reported melting point of the material that they isolated was 175 °C, close to the one we recorded, 173−174 °C, for **2a**; consequently, it seems likely that the product of this reaction was **2a**, 1-acyl-2-thiohydantoin. In 1993,[11] Marton et al. used Johnson's conditions to prepare compounds **2a** and **2c** and many other related compounds; they drew the correct structures and used the name that is now (IUPAC) accepted for them, i.e., 1-acyl-2-thiohydantoins. However, two other reports, one in 1987[12] and the other in 2002,[13] mention syntheses similar to that for compound **2a** but draw structure **3** and use the IUPAC name for that latter structure, i.e., 3-acyl-2-thiohydantoin. In both reports, this compound was an intermediate, and the acyl group was removed via hydrolysis, so the possible structural misassignment was of no great consequence to the authors. However, it is potentially important to researchers who use these reactions, and we feel that these structures were not correctly assigned. We postulate that the compound prepared in the 2002 report was **2a**, and not **3a**, based on the fact that the N*H* chemical shift given was 12.56 ppm in DMSO-d_6. Unfortunately, the 1987 paper gave NMR data obtained in $CDCl_3$/DMSO-d_6 mixtures. The N*H* chemical shift which appeared at 3.90 ppm was surprising since this signal was observed at such a high field. When the proton NMR of our sample of **2a** was recorded in (∼1 mL, 2:1) $CDCl_3$/DMSO-d_6, the N^3*H* was observed at 12.31 ppm. These values do not correspond well, but we think it is more likely that an incorrect chemical shift value was inadvertently reported in the 1987 paper than it is that they actually prepared a compound possessing structure **3a**.

Any one-step reaction that transforms amino acids into the types of heterocycles commonly found in pharmaceuticals is of interest in contemporary medicinal chemistry. Reaction 2 represents one such transformation, but potential practitioners should exercise care when interpreting the chemical literature in this area.

Experimental Section

Synthesis of 3-Benzyl-5-methyl-2-thiohydantoin (1). To a mixture of L-alanine methyl ester hydrochloride salt (0.500 g, 3.58 mmol) and Et_3N (0.5 mL, 3.58 mmol) in 10 mL of CH_2Cl_2 was slowly added benzyl isothiocyanate (0.475 mL, 3.58 mmol). The mixture was stirred for 1 h at 25 °C, after which a reflux condenser was attached. The temperature was raised to 40 °C, and stirring was continued for another 1 h. At this time, TLC revealed all of the benzylisothiocyanate has been consumed, and a new spot appeared. The solution was cooled to room temperature and the solution evaporated in vacuo. The residue was redissolved in 15 mL of CH_2Cl_2, washed with 10 mL of water and 10 mL of brine, and dried over Na_2SO_4. The colorless solution was concentrated and dried in vacuo to give **1** as a white, tiny crystalline material (0.410 g, 52%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.46 (s, 1H), 7.34−7.24 (m, 5H), 4.89 (d, J = 15.0 Hz, 1H), 4.84 (d, J = 15.0 Hz, 1H), 4.38 (q, J = 7.1 Hz, 1H), 1.29 (d, J = 7.1 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 182.2 (C), 175.6 (C), 136.5 (C), 128.5 (CH), 127.42 (CH), 127.40 (CH), 54.7 (CH), 43.7 (CH_2), 16.3 (CH_3). MS (ESI, *m*/*z*): 576 $(M + H)^+$. NMR data are in accordance with the literature.[2]

General Procedure for the Synthesis of Acyl-2-thiohydantoins. Synthesis of 1-Acetyl-2-thiohydantoin (2a). Glycine (1.0 g, 13.3 mmol) and NH_4SCN (1.038 g, 13.3 mmol) were ground together using a mortar and pestle. The mixed solid was transferred in a 50 mL round-bottom flask, acid anhydride (7.5 mL, 79.3 mmol) was added, and the mixture was heated in an oil bath at 100 °C for 30 min, by which time all solids were already dissolved. The light orange solution was poured into an ice/water mixture (20 mL) and stored in a freezer overnight. The resulting light orange solid was filtered and washed with cold water and dried under vacuum to afford **2a** as a light orange solid which appeared as a tiny crystalline-like material (1.08 g, 51%). Mp: 173−174 °C (lit.[7] mp 175−176 °C). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.58 (s, 1H), 4.40 (s, 2H), 2.68 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 182.5 (C), 170.4 (C), 169.3 (C), 52.2 (CH), 26.6 (CH_3). MS (ESI, *m*/*z*): 576 $(M + H)^+$.

1-Acetyl-5-methyl-2-thiohydantoin (2b). L-Alanine was used to prepare this material which followed the procedure for **2a**. After filtration and washing, the off-white solid was dried under vacuum to yield **2b** (1.30 g, 68%). Mp: 164−166 °C (lit.[9] 166 °C). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.63 (s, 1H), 4.67 (ddd, J = 7.1, 7.1, 7.1 Hz, 1H), 2.70 (s, 3H), 1.42 (d, J = 7.1 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 182.3 (C), 173.9 (C), 169.7 (C), 59.8 (CH), 27.4 (CH_3), 15.9 (CH_3). MS (APCI): 173 $(M + H)^+$.

1-Acetyl-5-benzyl-2-thiohydantoin (2c). L-Phenylalanine was used to prepare this material which followed the procedure for **2a**. After filtration and washing, the white solid was dried under vacuum to afford **2c** (0.850 g, 71%). Mp: 168−169 °C (lit.[8] 170 °C). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.43 (s, 1H), 7.26 (m, 3H), 6.98 (m, 2H), 4.99 (dd, J = 5.9, 2.7 Hz, 1H), 3.38 (dd, J = 13.9, 5.9 Hz, 1H), 3.12 (dd, J = 13.8, 2.7 Hz, 1H), 2.69 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 182.3 (C), 172.6 (C), 170.02 (C), 134.2 (C), 129.2 (CH), 128.4 (CH), 127.3 (CH), 63.5 (CH), 34.4 (CH_2), 27.4 (CH_3). MS (APCI): 249 $(M + H)^+$.

General Procedure for Deacylation. Synthesis of 2-Thiohydantoin (4a). A suspension of **2a** (0.148 g, 0.94 mmol) and ∼5 mL of 3 M HCl in a microwave tube was sealed and heated under microwave irradiation using a CEM (Discover) microwave at 150 °C for 5 min. The resulting clear, yellow solution was extracted with 4 × 5 mL of EtOAc. The combined EtOAc extracts were dried over Na_2SO_4, concentrated, and dried under vacuum to give **4a** as a light orange solid (0.081 g, 74%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.66 (s, 1H), 9.86 (s, 1H), 4.08 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 183.4 (C), 174.6 (C), 50.3 (CH_2). NMR data matched those reported in the literature.[14]

5-Methyl-2-thiohydantoin (4b). Compound **2b** was used to prepare the title compound, which followed the above procedure. After drying of the almost colorless solution, **4b** appear as white solid (66 mg, 87%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.64 (s,

(9) Johnson, T. B. *J. Biol. Chem.* **1912**, *11*, 97−101.
(10) Thielemann, H. *Z. Chem.* **1978**, *18*, 174.
(11) Marton, J.; Enisz, J.; Hosztafi, S.; Timar, T. *J. Agric. Food Chem* **1993**, *41*, 148−152.
(12) Villemin, D.; Ricard, M. *Synth. Commun.* **1987**, *17*, 283−9.
(13) Davis, R. A.; Aalbersberg, W.; Meo, S.; Moreira da Rocha, R.; Ireland, C. M. *Tetrahedron* **2002**, *58*, 3263−3269.
(14) Osz, E.; Szilagyi, L.; Marton, J. *J. Mol. Struct.* **1998**, *442*, 267−274.

1H), 10.01 (s, 1H), 4.23 (dddd, $J = 1.1, 7.1, 7.1, 7.1$ Hz, 1H), 1.24 (d, $J = 7.1$ Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 182.0 (C), 177.3 (C), 56.3 (CH), 16.1 (CH_3). NMR data matched those reported in the literature.[14]

5-Benzyl-2-thiohydantoin (4c). Compound **2c** was used to prepare the title compound which followed the above procedure. After drying of the colorless liquid, **4c** appear as white solid (0.35 g, 84%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.44 (s, 1H), 10.07 (s, 1H), 7.27 (m, 2H), 7.22 (m, 1H), 7.17 (m, 2H), 4.56 (t, $J = 5.1$ Hz, 1H), 2.98 (dd, $J = 5.0, 2.8$ Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 182.3 (C), 175.8 (C), 135.0 (C), 129.7 (CH), 128.3 (CH), 127.0 (CH), 61.5 (CH), 35.7 (CH_2). NMR data matched those reported in the literature.[14]

Synthesis of 5-Acetyl-4-thiohydantoic Acid Methyl Ester (5). To a mixture of glycine methyl ester hydrochloride salt (0.50 g, 3.98 mmol) and Et_3N (0.350 mL, 3.98 mmol) in 15 mL of dry CH_2Cl_2 was slowly added acyl isothiocyanate (0.559 mL, 3.98 mmol). The mixture was stirred for 1 h at 25 °C, after which a reflux condenser was attached. The temperature was raised to 40 °C, and stirring was continued for another 1 h. After 1 h, a TLC of the reaction showed a faint major spot ($R_f = 0.41$, 40% EtOAc/hexanes). The red brown mixture was cooled to room temperature and then evaporated in vacuo. The residue was redissolved in 15 mL of CH_2Cl_2, washed with 10 mL water and 10 mL brine, and dried over Na_2SO_4. The solution was concentrated and dried in vacuo to give **5** as an off white solid (0.475 g, 75%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.36 (s, 1H), 10.85 (t, $J = 5.$ 7 Hz, 1H), 4.37 (d, $J = 5.7$ Hz, 2H), 3.66 (s, 3H), 2.09 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 181.2 (C), 172.3 (C), 169.0 (C), 52.1 (CH_2), 46.2 (CH_3), 23.8 (CH_3). HRMS (ESI): calcd for $C_6H_{10}N_2O_3S$ 191.0412 $(M + H)^+$, found 191.0497.

Acknowledgment. Support for this work was provided by the NIH (MH 070040) and by the Robert A. Welch Foundation. The TAMU/LBMS-Applications Laboratory, directed by Dr. Shane Tichy, is also thanked.

Note Added after ASAP Publication. The general procedure for the synthesis of compound **2a** was incomplete in the version published ASAP February 14, 2006; the corrected version was published ASAP February 16, 2006.

Supporting Information Available: NMR spectra (^{1}H, ^{13}C, and COSY) of compounds **1**, **2a**−**c**, **4a**−**c**, and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO052576I