New Heterocycles from the Reaction between Some Natural α-Amino Acid Hydrazides and Formaldehyde

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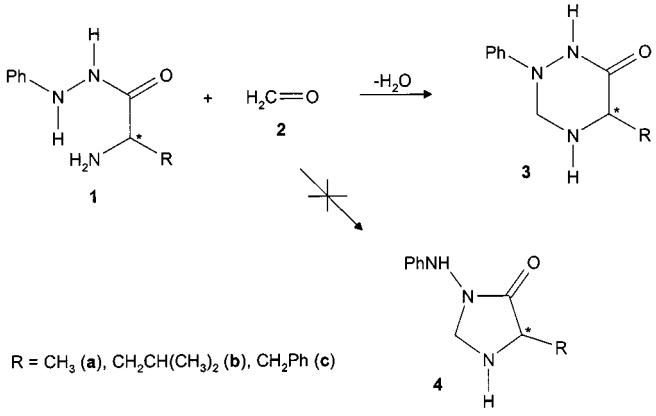
The ring forming condensation between some natural α-amino acid phenylhydrazides (**1**) and aqueous formaldehyde (**2**) has opened a novel synthetic route to hexahydro-1,2,4-triazin-6-one derivatives (**3**). Polycyclic systems were obtained from the same reaction carried out with L-aspartic acid 1,4-bis(2-phenylhydrazide) (**1d**), L-histidine phenylhydrazide (**1e**) and L-tryptophan phenylhydrazide (**1f**) which gave perhydro-4,6-dioxo-2,8-diphenyl[1,2,4]triazino[4,5-*d*]-[1,2,4]triazepine (**5**) perhydro-1-oxo-3-phenylimidazo[5,4-*d*][1,2,4]triazino[4,5-*a*]pyridine (**7**) and 1,2,3-*H*-3-(2-phenylcarbazoyl)-β-carboline (**8**), respectively. Substrates **1** were conveniently obtained by direct reaction of phenylhydrazine with L-α-amino acid esters retaining the original chirality.

Combining our recent interest in some aspects of the chemistry of hydrazides[1] and our long standing research in the reactions of formaldehyde with amines,[2] we investigated the reaction of a few hydrazides of natural amino acids with aqueous formaldehyde, a study which does not appear to have precedent in the chemical literature.

Results and Discussion

When phenylhydrazides of L-amino acids such as alanine, leucine and phenylalanine (**1a**−**c**) were allowed to react with formaldehyde, the expected hexahydro-1,2,4-triazin-6-ones (**3a**−**c**) were isolated in good to moderate yields (Scheme 1).



Scheme 1

Since monomeric and oligomeric *N*-methylene derivatives of **1** have the same elemental composition as **3**, the presence of a ring containing three nitrogen atoms had to be carefully deduced. The absence of resonance in the ^{13}C-NMR spectra for double bonded methylene group confirmed that the monomeric imine structure did not form.

The oligomeric structures of **1** were excluded by considering the ^{1}H-NMR features of the NHNH(Ar) skeleton: these two protons usually showed up in the range δ = 6−10, as two singlets.[1] In our products one of the proton resonances for the hydrazine function was missing in the range δ = 6−10, whereas an amino proton showed up at δ = 2−3 as a broad singlet. Both facts are good evidence for the cyclic structure **3** or **4**.

Absence of both the parent ions for oligomeric products of the imines (which are usually very weak) and any deoligomerization ions were partial evidence for the absence of such products. Moreover, hydrazides previously investigated,[1] and those prepared during this work, showed a very intense ion at *m*/*z* = 108, corresponding to the composition $C_6H_8N_2$, which was poorly represented in 1-phenyl-1-alkyl hydrazides[1] and in two of the products prepared (**3a** and **3c**). Compound **3b** actually showed such a peak, but most likely it originated from the involvement of the side chain during fragmentation. On the other hand, a common feature in the MS spectra of **3a**−**c** was the presence of an intense peak at *m*/*z* = 120 which likely corresponds to the composition C_7H_7N.

We were then left with the isomeric structures **3** and **4** (Scheme 1) for the definitive identification of the separated products. The well-known reductive cleavage of N−N bonds by Raney nickel[3] proved to be effective on phenylhydrazide **1a**, yielding aniline and 2-aminopropanamide as confirmed by GC-MS; moreover, the same reaction carried out on **3a** was ineffective even for prolonged reaction periods. Furthermore, silylation of **3a** yielded a monotrimethylsilyl derivative, an important indication of the presence of a single amino nitrogen as in **3**. Analogously, the reaction of this compound with acetyl chloride gave a monoacetyl derivative, while phenylhydrazine undergoes double acetylation under the same conditions.[4] All these results were consistent with a structure like **3** but not **4**.

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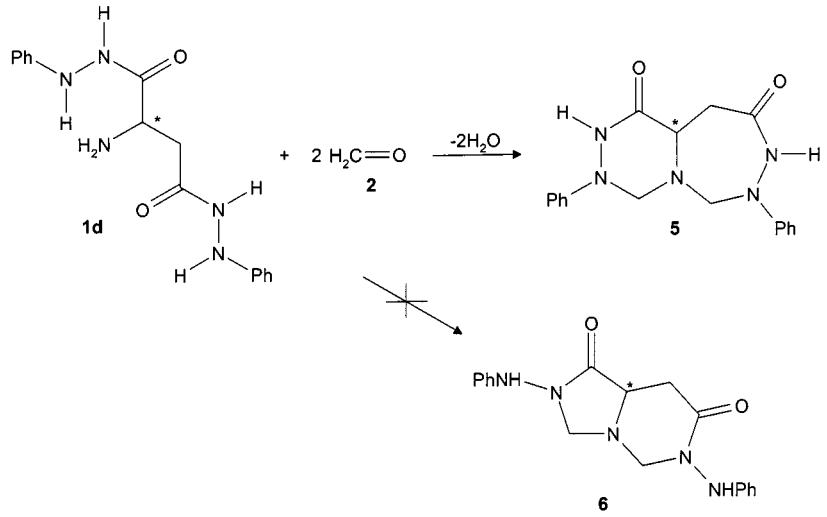


Scheme 2

Some further spectroscopic features of **3** are noteworthy. A notable feature in NMR spectra of **3a−c** is the non-equivalence of $N-CH_2-N$ protons, likely due to the diastereotopic effect of the α-chiral centre: a doublet of doublets was thus detected instead of a singlet. In the case of **1b** and **3b**, the diastereotopic effect was observed also for the geminal methyl groups.

Hexahydro-1,2,4-triazin-6-ones represent interesting examples[5] of derivatives of 1,2,4-triazines, for which an extensive research concerning both synthetic methods and biological activity is documented. Although the most frequently reported methods of their preparation involve a [4 + 2] cyclization, where hydrazines play the role of the two centre component,[6] a recent work reported the [5 + 1] cyclization performed by the reaction of amino acid hydrazides and orthocarboxylates.[7] No straightforward method is yet available for the preparation of hexahydro-1,2,4-triazin-6-ones, and very few examples of simple derivatives can be found in the literature.[8][9]

Under similar experimental conditions, L-aspartic acid 1,4-bis(2-phenylhydrazide) (**1d**) reacted with two molecules of formaldehyde affording an interesting triazine ring fused with a seven-membered heterocycle, namely perhydro-4,6-dioxo-2,8-diphenyl[1,2,4]triazino[4,5-*d*][1,2,4]triazepine (**5**) (Scheme 2).

The isomeric structure **6**, a 5/6 rings combination instead of 6/7, was excluded because the treatment with Raney nickel left the compound unchanged even after long reaction times.

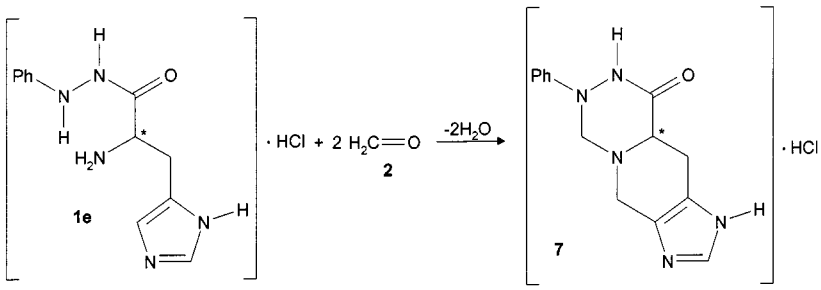
The spectroscopic data are in agreement with the structure proposed for **5**. An interesting feature observed in the ^{1}H-NMR spectra is the non-equivalence of all six methylene protons, each appearing with a geminal coupling constant, likely due to a diastereotopic effect. Two singlets were present at δ = 9.9 and 10.2 for the two hydrazide protons in DMSO. Some examples of compounds in the class of fused rings involving 1,2,4-triazepines have been reported,[10] nevertheless there are no reports either on perhydro-4,6-dioxo-2,8-diphenyl[1,2,4]triazino[4,5-*d*][1,2,4]-triazepine (**5**) or their analogues. A route similar to our [6+1] atom combination has been employed in the preparation of 1,3,4-benzotriazepine system.[10]

A different behaviour was observed for heteroaromatic amino acid phenylhydrazides such as L-histidine and L-tryptophan phenylhydrazide (**1e** and **1f**), whose reactivity was strongly conditioned by the presence of an imidazole and an indole group, respectively. The hydrochlorides of **1e** and **1f** gave complex mixtures under the same experimental conditions employed for **1a−c**: in both cases a single product was obtained by heating the reaction mixture in DMSO. The presence of both HCl and DMSO turned out to be necessary for a good outcome to the reactions, providing a catalytic environment for the complete conversion of the intermediates. L-Histidine phenylhydrazine hydrochloride (**1e**) yielded a triazine ring fused with a six-membered heterocycle, namely perhydro-1-oxo-3-phenylimidazo[5,4-*d*][1,2,4]triazino[4,5-*a*]pyridine (**7**), isolated as a hydrochloride (Scheme 3).

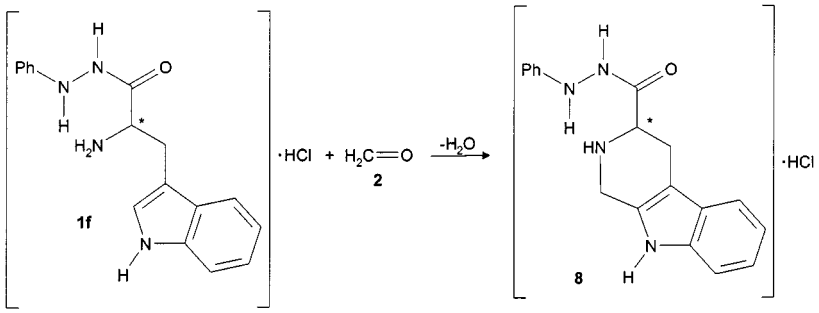
The structure of **7** was determined by NMR: the ^{13}C-NMR spectrum, if compared with that of the starting hydrazide **1e**, showed a quaternary carbon, instead of a primary one, in the region of aromatic signals, and the ^{1}H NMR revealed the absence of a singlet at δ = 7.0 in DMSO, due to an imidazole CH. This is a good indication that formaldehyde was responsible for an electrophilic attack on the imidazole ring.

Cyclization reactions like this one were reported for histamine and histidine under acidic conditions.[11] As already observed for **5**, all the six methylene protons were non-equivalent, each showing up with a geminal coupling constant.

In the case of L-tryptophan phenylhydrazide hydrochloride, the electrophilic substitution on the reactive 2-position of the indole ring upon treatment with formaldehyde was preferred to the ring closure to 1,2,4-triazine system. No triazine was formed, but 1,2,3-*H*-3-(2-phenylcarbazoyl)-β-



Scheme 3



Scheme 4

carboline (**8**) was isolated as the hydrochloride (Scheme 4). This behaviour suggested that the initial reaction of formaldehyde with **1** occurred at the amino group.

Compounds containing the heterocyclic skeleton of perhydro-1-oxo-3-phenylimidazo[5,4-*d*][1,2,4]triazino[4,5-*a*]pyridine (**7**) have not been reported so far. Conversely, many derivatives containing the β-carboline moiety present in 1,2,3-*H*-3-(2-phenylcarbazoyl)-β-carboline (**8**), have been extensively described in the literature. This subunit was found in many naturally occurring compounds, subjected to intensive chemical and biochemical research[12] due to their pharmacological importance. The β-carboline system containing the pyridine ring in a reduced state has received special attention because it is the starting point for several alkaloids.[13]

In the cases **1a**, **3a**, **1b**, **3b**, **1c**, and **3c** where the stereochemistry of the products was established, the enantiomeric purity of the original L-α-amino acids was retained. These compounds with rigid frameworks containing a natural amino acid moiety could be attractive targets in the design of new peptidomimetic structures with biological activity.

As a closing remark we wish to point out the straightforward preparation of L-amino acid phenylhydrazides (**1**) by the simple condensation of the appropriate L-amino acid esters and an excess of neat phenylhydrazine at 60°C: neither a catalyst nor the protection of the amino group was required.[14] This observation may find an application in the chemistry of peptide synthesis where the phenylhydrazide protecting group has received recent attention for its easy removal.[15] It is quite interesting to notice that the NMR spectra of phenylhydrazides **1a**−**e** did not show the (*E*/*Z*) isomerism reported for other hydrazides:[1][16] in solution these compounds were present in one conformation only. The sole exceptions were **1f** and **8**, whose spectra in DMSO at 40°C revealed the presence of two isomers: their signals merged at 100°C, thus confirming the existence of a conformational equilibrium

Experimental Section

General: All reagents were of commercial quality (Aldrich, Fluka) and were used without further purification. − GC-MS analyses were performed with a Fisons TRIO 2000 gas-chromatograph−mass spectrometer, working in the positive ion 70 eV electron impact mode. Spectra were recorded in the range 35−450 u. Injector temperature was kept at 250°C and the column (Supelco SPB™-5, 30 m long, 0.25 mm i.d.) temperature was programmed from 60°C to 300°C with a gradient of 10°C/min. − The enantiomeric purity of **1a**, **3a**, **1b**, **3b**, **1c**, and **3c** was determined using chiral GC analysis, upon comparison with the corresponding D,L mixtures (the column was a Chrompack 7495 WCOT, liquid phase Chirasil-val-L, i.d. 0.22 mm, o.d. 0.35 mm). − Optical rotations were determined at 21°C using a POLAX-D polarimeter purchased from ATAGO (Japan). − IR spectra were obtained with a Nicolet FT-IR Magna 550 spectrophotometer using KBr technique for solids and recorded in the range 4000−400 cm^{-1}. − ^1H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ at room temperature on a Bruker AC-F 200 spectrometer at 200 and 50 MHz, respectively. NMR peak locations are reported as δ-values from TMS (^1H NMR) and the central peak of $CDCl_3$ (^{13}C NMR). − Elemental analyses were performed with a Carlo Erba Mod. 1106 elemental analyzer. − Melting points were determined with an automatic Mettler (Mod. FP61) apparatus and are not corrected.

Preparation of the L-α-Amino Acid Phenylhydrazides 1a−c: The appropriate L-α-amino acid methyl ester hydrochloride (9.3 mmol) and freshly distilled phenylhydrazine (5.0 mL, 46.5 mmol) were mixed in a flask fitted with a condenser and a magnetic stirring bar. The mixture was stirred at 60°C for 16 h under inert atmos-

phere. The reaction was allowed to cool down to room temperature and CH_2Cl_2 (100 mL), $Na_2CO_3 \cdot 10\,H_2O$ (11 g), and H_2O (ca. 3 mL) were added. The mixture was vigorously stirred for 3 h. After filtration of salts the solvent was reduced, and the unchanged phenylhydrazine was removed under reduced pressure (60°C at ca. 0.5 mbar). After addition of Et_2O (20 mL) and ca. 3 mL of hexane, the mixture was stirred for 3 h and the solid product was filtered and washed with Et_2O. **1a** was obtained in spectroscopically pure form; **1b** and **1c** were recrystallised from *i*PrOH/hexane.

L-Alanine Phenylhydrazide (1a): Yield 85%, m.p. 116°C. − IR (KBr): $\tilde{\nu}$ = 3284 cm^{-1}, 3203, 1662, 1600, 1533, 1497. − MS (70 eV); *m*/*z* (%): 179 [M^+] (41), 162 (47), 134 (35), 109 (10), 108 (100), 107 (14), 105 (6), 93 (17), 92 (17), 77 (17), 65 (7). − ^1H NMR ($CDCl_3$): δ = 1.40 (d, 3 H, *J* = 6.5 Hz), 1.54 (broad s, 1 H), 3.61 (q, 1 H, *J* = 6.5 Hz), 6.14 (broad s, 1 H), 6.75−6.95 (m, 3 H), 7.15−7.30 (m, 2 H). − ^{13}C NMR ($CDCl_3$): δ = 21.7, 50.2, 113.5, 121.1, 129.1, 147.9, 175.1. − $[\alpha]_D$ = +3.8 (*c* = 0.17, MeOH). − $C_9H_{13}N_3O$ (179.2): calcd. C 60.32, H 7.31, N 23.45; found C 60.55, H 7.52, N 23.56. Registry no.: 97458−28−1.

L-Leucine Phenylhydrazide (1b): Yield 70%, m.p. 148°C (ref.[14c] 122°C). − IR (KBr): $\tilde{\nu}$ = 3238 cm^{-1}, 2958, 1650, 1603, 1500. − MS (70 eV); *m*/*z* (%): 221 [M^+] (24), 204 (17), 134 (16), 109 (10), 108 (100), 107 (15), 93 (54), 92 (30), 86 (95), 77 (22), 65 (13). − ^1H NMR ($CDCl_3$): δ = 0.93 (d, 3 H, *J* = 6.3 Hz), 0.97 (d, 3 H, *J* = 6.1 Hz), 1.30−1.60 (m, 3 H), 1.70−1.90 (m, 2 H), 3.53 (dd, 1 H, CH, *J* = 9.8, 4.0 Hz), 6.10 (broad s, 1 H), 6.75−6.95 (m, 3 H), 7.20−7.30 (m, 2 H). − ^{13}C NMR ($CDCl_3$): δ = 21.3, 23.3, 24.7, 44.0, 53.0, 113.6, 121.1, 129.1, 148.0, 175.0. − $[\alpha]_D$ = +20.7 (*c* = 0.20, MeOH). − $C_{12}H_{19}N_3O$ (221.3): calcd. C 65.13, H 8.65, N 18.99; found C 65.33, H 8.88, N 19.20. Registry no.: 6278−97−3.

L-Phenylalanine Phenylhydrazide (1c): Yield 80%, m.p. 147°C (ref.[14c] 134°C). − IR (KBr): $\tilde{\nu}$ = 3291 cm^{-1}, 3223, 1684, 1656, 1604. − MS (70 eV); *m*/*z* (%): 255 [M^+] (30), 238 (10), 164 (53), 147 (11), 134 (26), 131 (32), 120 (100), 108 (43), 103 (21), 93 (25), 92 (26), 91 (27), 77 (23), 65 (20). − ^1H NMR ($CDCl_3$): δ = 2.75 (dd, 1 H, *J* = 13.5, 7.0 Hz), 2.95 (dd, 1 H, *J* = 13.5, 6.5 Hz), 3.55 (t, 1 H, *J* = 6.5 Hz), 6.55 (d, 2 H, *J* = 8.2 Hz), 6.67 (t, 1 H, *J* = 7.0 Hz), 7.07 (t, 2 H, *J* = 7.6 Hz), 7.20−7.40 (m, 5 H), 7.62 (broad s, 1 H). − ^{13}C NMR ($CDCl_3$): δ = 41.5, 55.2, 112.1, 118.3, 126.1, 128.1, 128.5, 129.3, 138.5, 149.1, 174.1. − $[\alpha]_D$ = +37.1 (*c* = 0.27, MeOH). − $C_{15}H_{17}N_3O$ (255.3): calcd. C 70.56, H 6.71, N 16.46; found C 70.67, H 6.82, N 16.64. Registry no.: 14723−88−7.

Preparation of L-Aspartic Acid 1,4-Bis(2-phenylhydrazide) (1d): The L-aspartic acid methyl ester hydrochloride (3.9 g, 20.0 mmol) was neutralised with an aqueous saturated solution of Na_2CO_3 (200 mL), pH 10−11, and extracted with AcOEt (2 × 200 mL). The free amino acid ester was obtained (2.6 g, 16.0 mmol) after accurate solvent removal (at ca. 0.5 mbar, 50°C). Freshly distilled phenylhydrazine (8.6 mL, 80.0 mmol) was added and the mixture was stirred at 90°C for 24 h under inert atmosphere in a flask fitted with a condenser. The unchanged phenylhydrazine was distilled under reduced pressure (60°C at ca. 0.5 mbar) and the residue was treated with 50 mL Et_2O and ca. 3 mL of hexane. The solid was filtered, washed with Et_2O and recrystallised from MeOH/*i*PrOH/hexane (85%), m.p. (dec.) = 178°C. − IR (KBr): $\tilde{\nu}$ = 3230 cm^{-1}, 1645, 1605, 1496. − MS (70 eV); *m*/*z* (%): 313 [M^+] (3), 296 (4), 205 (14), 177 (24), 161 (14), 134 (8), 108 (100), 107 (17), 106 (18), 105 (14), 93 (76), 92 (48), 91 (22), 78 (22), 77 (60), 70 (64), 65 (38). − ^1H NMR ([D_6]DMSO): δ = 2.37 (dd, 1 H, *J* = 14.7, 8.8 Hz), 2.52 (dd, 1 H, *J* = 14.7, 4.7 Hz), 3.30 (broad s, 2 H), 3.69 (dd, 1 H, *J* = 8.8, 4.7 Hz), 6.60−6.80 (m, 6 H), 7.00−7.20 (m, 4 H), 7.60 (s, 1 H), 7.63 (s, 1 H), 7.80 (s, 1 H), 9.00 (s, 1 H). − ^{13}C NMR ([D_6]DMSO): δ = 39.0, 50.9, 112.2, 118.3, 118.4, 128.5, 149.2, 170.1, 173.8. − $C_{16}H_{19}N_5O_2$ (313.3): calcd. C 61.33, H 6.11, N 22.35; found C 61.57, H 6.28, N 22.51.

Preparation of L-Amino Acid Phenylhydrazides Hydrochloride (1e−f): L-Histidine methyl ester dihydrochloride or L-tryptophan methyl ester hydrochloride (16.3 mmol) and freshly distilled phenylhydrazine (9.0 mL, 82.0 mmol) were mixed in a flask fitted with a condenser and a magnetic stirring bar. The mixture was stirred at 90°C for 6 h under inert atmosphere. After the addition of 100 mL of *i*PrOH, the solid was filtered. **1e** was recrystallised from *i*PrOH/MeOH under stirring (which prevented the precipitation of the product in the form of a gel). Compound **1f** was crystallised from MeOH/*i*PrOH/Et_2O. The product was hygroscopic. Potentiometric titration of chloride with a silver nitrate solution[17] in the presence of HNO_3 confirmed that **1e** and **1f** were isolated as the monohydrochlorides.

L-Histidine Phenylhydrazide Hydrochloride (1e): Yield 50%, m.p. (dec.) = 195°C. − IR (KBr): $\tilde{\nu}$ = 3484 cm^{-1}, 3414, 3257, 3224, 1707, 1606, 1497. − MS (70 eV); *m*/*z* (%): 245 [M^+] (17), 228 (2), 164 (6), 138 (6), 123 (7), 121 (10), 110 (100), 108 (23), 93 (32), 92 (17), 82 (46). − ^1H NMR ([D_6]DMSO): δ = 3.00−3.15 (m, 2 H), 4.10 (t, 1 H, *J* = 5.8 Hz), 6.50−7.20 (m, 6 H), 7.70 (s, 1 H), 7.80 (s, 1 H). − ^{13}C NMR ([D_6]DMSO): δ = 29.4, 51.8, 112.3, 116.0, 118.5, 128.5, 132.9, 135.0, 148.6, 168.9. − $C_{12}H_{16}ClN_5O$ (281.7): calcd. C 51.16, H 5.72, N 24.86; found C 50.18, H 6.17, N 23.72.

L-Tryptophan Phenylhydrazide Hydrochloride (1f): Yield 60%, m.p. (dec.) = 200°C. − IR (KBr): $\tilde{\nu}$ = 3305 cm^{-1}, 3227, 1683, 1603, 1495, 1481. − MS (70 eV); *m*/*z* (%): 306 [M^+] (10), 294 (60), 277 (18), 170 (80), 164 (77), 163 (37), 159 (60), 158 (38), 143 (41), 132 (44), 130 (100), 117 (29), 115 (25), 108 (35), 93 (80), 92 (58), 77 (65). − ^1H NMR ([D_6]DMSO, 100°C): δ = 3.30 (d, 2 H, *J* = 7.2 Hz), 4.17 (t, 1 H, *J* = 7.3 Hz), 6.50−7.90 (m, 10 H), 11.10 (broad s, 1 H). − ^{13}C NMR ([D_6]DMSO, 100°C): δ = 26.6, 51.7, 106.8, 111.0, 112.4, 118.0, 118.5, 120.5, 124.2, 126.9, 128.0, 136.0, 147.9, 167.6. − ^{13}C NMR ([D_6]DMSO, 45°C; the two (*E*/*Z*) isomers, indicated with A and B, were present in ca. 1:12 ratio): δ = 26.3 (A), 27.1 (B), 50.1 (A), 51.7 (B), 107.0 (B), 111.3 (B), 112.4 (B), 112.6 (A), 118.2 (A), 118.4 (B), 118.6 (B), 119.7 (A), 121.0 (B), 124.6 (B), 125.0 (A), 126.9 (A), 127.0 (B), 127.9 (A), 128.4 (B), 128.9 (A), 136.2 (B), 136.4 (A), 168.1 (B), 172.9 (A). − $C_{17}H_{19}ClN_4O$ (330.8): calcd. C 61.8, H 5.76, N 17.0; found C 61.27, H 5.82, N 16.70.

Reaction of L-Alanine Phenylhydrazide (1a) with Raney Nickel: A well stirred mixture of **1a** (0.11 g, 0.6 mmol) and Raney nickel (ca. 0.08 g) in absolute EtOH (5 mL) was hydrogenated at 60°C at atmospheric pressure during 7 h. The reaction mixture was diluted with MeOH (10 mL) and, after removal of the catalyst, the solvents were distilled off under reduced pressure. The residue was washed with Et_2O. The ether extract contained aniline formed during the reaction (0.05 g, 0.5 mmol, 89%); the solid residue consisted mainly of 2-aminopropanamide (0.04 g, 0.48 mmol, 80%) identified by comparison with an authentic specimen, prepared according to a described procedure.[18]

Preparation of Hexahydro-1,2,4-triazin-6-ones (3a−c): A solution of the appropriate L-amino acid phenylhydrazide (**1a−c**) (4.5 mmol) and aqueous formaldehyde (36%, 0.37 mL, 4.8 mmol) in absolute EtOH (45 mL) was stirred at 40°C for 3 h. After solvent removal under reduced pressure, the solid residue was purified: **3a** was recrystallised from AcOEt/hexane, **3c** from *i*PrOH/hexane, and **3b** was purified by a treatment with charcoal in EtOH for 5 min at room temp. These products could not be purified by absorption chromatography since they extensively decomposed upon contact on silica gel or alumina.

Hexahydro-5-methyl-2-phenyl-1,2,4-triazin-6-one (3a): Yield 60%, m.p. 147°C. − IR (KBr): $\tilde{\nu}$ = 3272 cm^{-1}, 3213, 1708, 1691, 1603, 1498. − MS (70 eV); *m/z* (%): 191 [M^+] (100), 162 (13), 134 (63), 120 (81), 119 (33), 108 (13), 107 (20), 105 (22), 93 (21), 92 (60), 91 (15), 77 (28), 65 (21). − ^{1}H NMR ($CDCl_3$): δ = 1.40 (d, 3 H, *J* = 6.7 Hz), 2.12 (broad s, 1 H), 3.56 (q, 1 H, *J* = 6.7 Hz), 4.38 (d, 1 H, *J* = 7.3 Hz), 4.45 (d, 1 H, *J* = 7.3 Hz), 6.44 (broad s, 1 H), 6.65−7.00 (m, 3 H), 7.15- 7.35 (m, 2 H). − ^{13}C NMR ($CDCl_3$): δ = 16.5, 53.8, 63.0, 113.0, 120.8, 129.0, 145.8, 175.4. − $[\alpha]_D$ = +59.2 (*c* = 0.42, MeOH). − $C_{10}H_{13}N_3O$ (191.2): calcd. C 62.81, H 6.85, N 21.97; found C 62.68, H 6.94, N 21.77.

Hexahydro-5-(2′-methylpropyl)-2-phenyl-1,2,4-triazin-6-one (3b): Yield 70%, m.p. 111°C. − IR (KBr): $\tilde{\nu}$ = 3247 cm^{-1}, 2958, 1710, 1603, 1497. − MS (70 eV); *m/z* (%): 233 [M^+] (63), 204 (6), 177 (36), 176 (30), 162 (12), 161 (14), 149 (28), 146 (16), 134 (45), 120 (100), 119 (53), 108 (74), 107 (22), 105 (17), 98 (20), 93 (53), 92 (76), 86 (67), 77 (26). − ^{1}H NMR ($CDCl_3$): δ = 1.00 (t, 6 H, *J* = 6.4 Hz), 1.35−1.60 (m, 1 H), 1.70−1.95 (m, 2 H), 2.10 (broad s, 1 H), 3.50 (dd, 1 H, *J* = 10.4, 3.8 Hz), 4.42 (d, 1 H, *J* = 7.3 Hz), 4.50 (d, 1 H, *J* = 7.3 Hz), 6.50 (s, 1 H), 6.65−6.90 (m, 3 H), 7.10−7.30 (m, 2 H). − ^{13}C NMR ($CDCl_3$) δ = 21.4, 23.2, 25.0, 40.4, 56.5, 63.5, 113.4, 121.2, 129.2, 146.0, 175.6. − $[\alpha]_D$ = −6.8 (*c* = 0.22, MeOH). − $C_{13}H_{19}N_3O$ (233.3): calcd C 66.92, H 8.21, N 18.01; found C 66.72, H 8.33, N 17.77.

Hexahydro-5-benzyl-2-phenyl-1,2,4-triazin-6-one (3c): Yield 85%, m.p. 154°C. − IR (KBr): $\tilde{\nu}$ = 3288 cm^{-1}, 1704, 1607, 1497, 1452. − MS (70 eV); *m/z* (%): 267 [M^+] (58), 238 (3), 176 (91), 147 (15), 134 (35), 132 (48), 131 (28), 120 (100), 119 (35), 106 (23), 105 (37), 104 (20), 93 (33), 92 (80), 91 (65), 77 (30), 65 (24). − ^{1}H NMR ($CDCl_3$): δ = 3.12 (dd, 2 H, *J* = 5.6, 2.0 Hz), 3.83 (t, 1 H, *J* = 5.3 Hz), 4.21 (d, 1 H, *J* = 7.0 Hz), 4.33 (d, 1 H, *J* = 7.0 Hz), 6.00 (broad s, 1 H), 6.55 (d, 2 H, *J* = 8.2 Hz), 6.90 (t, 1 H, *J* = 7.0 Hz), 7.15 (t, 2 H, *J* = 7.6 Hz), 7.20−7.35 (m, 5 H). − ^{13}C NMR ($CDCl_3$): δ = 36.6, 58.9, 63.8, 113.7, 121.6, 127.1, 128.7, 129.3, 129.8, 136.4, 145.9, 173.9. − $[\alpha]_D$ = +43.2 (*c* = 0.35, MeOH). − $C_{16}H_{17}N_3O$ (267.3): calcd C 71.89, H 6.41, N 15.72; found C 71.65, H 6.22, N 15.87.

Reaction of Hexahydro-5-methyl-2-phenyl-1,2,4-triazin-6-one (3a) with Raney Nickel: A well stirred mixture of **3a** (0.11 g, 0.57 mmol) and Raney nickel (ca. 0.07 g) in absolute EtOH (5 mL) was hydrogenated at 60°C at atmospheric pressure during 24 h. GC-MS and TLC analysis of the reaction mixture showed the presence only of the starting material **3a**. This was confirmed by ^{1}H- and ^{13}C-NMR analysis of the solid (0.10 g, 0.52 mmol, 91%) obtained after removal of the catalyst and solvent.

Silylation of Hexahydro-5-methyl-2-phenyl-1,2,4-triazin-6-one (3a): A solution of **3a** (0.04 g, 0.21 mmol) and a mixture of BSTFA and TMCS (99:1; 0.3 mL) in pyridine (0.30 mL) was heated at 60°C during 90 min in a sealed tube. GC-MS analysis of the reaction mixture revealed the presence of two compounds: unchanged **3a** and its monosilyl derivative [MS (70 eV); *m/z* (%): 263 [M^+] (35), 234 (33), 219 (15), 191 (4), 178 (5), 150 (16), 129 (25), 73 (100), 56 (20)].

Acetylation of Hexahydro-5-methyl-2-phenyl-1,2,4-triazin-6-one (3a): A solution of acetyl chloride (0.04 mL, 0.56 mmol) and **3a** (0.025 g, 0.13 mmol) in pyridine (1.0 mL) was stirred at 40°C during 40 min in a sealed tube. GC-MS analysis of the neutralised reaction mixture showed the presence of the monoacetyl derivative of **3a** [MS (70 eV); *m/z* (%): 233 [M^+] (29), 192 (26), 191 (100), 162 (18), 134 (18) 120 (20) 93 (4), 92 (7), 65 (15), 57 (15), 56 (39), 44 (12), 43 (26)] as the sole product.

Preparation of Perhydro-1-oxo-3-phenylimidazo[5,4-*d*][1,2,4]triazino[4,5-*a*]pyridine Hydrochloride (7): A solution of **1e** (2.0 g, 7.1 mmol) and aqueous formaldehyde (36%, 1.2 mL, 15.6 mmol) in 200 mL MeOH was stirred at 50°C for 3 h. After solvent removal under reduced pressure, 10 mL of DMSO were added and the solution was heated at 40°C for 3 h. The solvent was distilled at ca. 0.5 mbar, the residue dissolved in the minimum amount of MeOH and the solution was added dropwise to AcOEt under stirring in order to let a white solid precipitate. It was filtered and obtained in spectroscopically pure form (80%), m.p. (dec.) = 220°C. − IR (KBr): $\tilde{\nu}$ = 3420 cm^{-1}, 3143, 1696, 1648, 1602, 1497. − MS (70 eV); *m/z* (%): 269 [M^+] (75), 240 (6), 212 (10), 177 (7), 162 (6), 134 (100), 122 (48), 120 (41), 108 (17), 107 (43), 95 (30), 93 (25), 92 (18), 77 (12), 36 (7). − ^{1}H NMR ([D_6]DMSO): δ = 2.52 (t, 1 H, *J* = 11.0 Hz), 3.08 (dd, 1 H, *J* = 15.2, 4.0 Hz), 3.48 (dd, 2 H, *J* = 11.1, 4.1 Hz), 3.83 (d, 1 H, *J* = 14.0 Hz), 4.22 (d, 1 H, *J* = 11.7 Hz), 4.83 (d, 1 H, *J* = 12.3 Hz), 6.80−7.50 (m, 5 H), 8.88 (s, 1 H), 10.30 (s, 1 H). − ^{13}C NMR ([D_6]DMSO): δ = 23.8, 44.9, 59.6, 69.7, 117.4, 121.7, 124.9, 125.0, 128.7, 132.3, 150.0, 167.4. − $C_{14}H_{16}ClN_5O$ (305.7): calcd C 54.99, H 5.27, N 22.90; found C 52.43, H 5.34, N 21.24.

Preparation of Perhydro-4,6-dioxo-2,8-diphenyl[1,2,4]triazino[4,5-*d*]-[1,2,4]triazepine (5): A solution of **1d** (1.4 g, 4.5 mmol) and aqueous formaldehyde (36%, 1.7 mL, 22.7 mmol) in EtOH (100 mL) was stirred at 40°C for 2 h. The product precipitated and was filtered. Compound **5** was obtained in spectroscopically pure form (70%), m.p. 168°C. − IR (KBr): $\tilde{\nu}$ = 3229 cm^{-1}, 3170, 1727, 1673, 1655, 1599, 1497. − MS (70 eV); *m/z* (%): 337 [M^+] (17), 308 (11), 230 (23), 217 (22), 201 (25), 188 (22), 174 (13), 161 (34), 147 (16), 121 (13), 120 (14), 108 (22), 106 (31), 105 (88), 104 (50), 93 (48), 92 (39), 91 (30), 77 (100), 65 (23). − ^{1}H NMR ([D_6]DMSO): δ = 2.09 (t, 1 H, *J* = 12.0 Hz), 2.67 (d, 1 H, *J* = 13.5 Hz), 3.30−3.60 (m, 1 H), 3.90 (d, 1 H, *J* = 13.5 Hz), 4.20 (d, 1 H, *J* = 12.1 Hz), 4.80 (d, 1 H, *J* = 12.1 Hz), 4.97 (d, 1 H, *J* = 13.5 Hz), 6.50−6.75 (m, 3 H), 6.80−7.10 (m, 5 H), 7.20−7.35 (m, 2 H), 9.80 (s, 1 H), 10.20 (s, 1 H). − ^{13}C NMR ([D_6]DMSO): δ = 60.4, 67.5, 69.2, 113.2, 117.6, 118.8, 121.9, 128.5, 128.6, 146.7, 150.2, 167.3, 175.5. − $C_{18}H_{19}N_5O_2$ (337.3): calcd. C 64.08, H 5.68, N 20.76; found C 64.34, H 5.43, N 21.02.

Reaction of Perhydro-4,6-dioxo-2,8-diphenyl[1,2,4]triazino[4,5-*d*]-[1,2,4]triazepine (5) with Raney Nickel: A well-stirred mixture of **5** (0.20 g, 0.59 mmol) and Raney nickel (ca. 0.07 g) in absolute EtOH (7 mL) was hydrogenated at 60°C at atmospheric pressure during 24 h. TLC analysis of the reaction mixture showed the presence only of the starting material **5**. This was confirmed by ^{1}H- and ^{13}C-NMR analysis of the solid (0.19 g, 0.56 mmol, 95%) obtained after removal of the catalyst and solvent.

Preparation of 1,2,3-H-3-(2-phenylcarbazoyl)-β-carboline Hydrochloride (8): A solution of **1f** (2.0 g, 6.1 mmol) and aqueous formaldehyde (36%, 1.0 mL, 13.4 mmol) in 170 mL MeOH was stirred at room temp. for 17 h. After a treatment with charcoal at room temp. (for at least 5 min) and solvent removal under reduced pressure, 10 mL of DMSO were added and the solution was heated at 50°C for 3 h. The solvent was distilled at ca. 0.5 mbar, the residue dissolved in the minimum amount of *i*PrOH and the solution was added dropwise to AcOEt under stirring in order to let a white solid precipitate. It was filtered and obtained in spectroscopically pure form **8** (73%), m.p. (dec.) = 215°C. − IR (KBr): $\tilde{\nu}$ = 3400 cm^{-1}, 3215, 1690, 1603, 1499. − MS (70 eV); *m/z* (%): 318 [M^+] (13), 215 (13), 211 (33), 183 (24), 171 (50), 170 (30), 169 (71), 168 (100), 167 (42), 144 (65), 143 (88), 140 (26), 130 (30), 93 (75), 77 (18), 66 (32). − ^{1}H NMR ([D_6]DMSO, 100°C, the sample should

be very dilute): δ = 3.00−3.52 (m, 2 H), 4.25−4.55 (m, 3 H), 6.70−7.55 (m, 9 H), 10.38 (s, 1 H), 11.01 (s, 1 H). − ^{13}C NMR ([D$_6$]DMSO, 100°C): δ = 23.1, 40.4, 54.1, 104.4, 111.1, 112.5, 117.8, 118.7, 121.3, 125.7, 126.3, 128.3, 136.3, 148.6, 168.2. − ^{13}C NMR ([D$_6$]DMSO, 45°C; the two (*E*/*Z*) isomers, indicated with A and B, were present in ca. 1:12 ratio): δ = 22.4 (A), 23.2 (B), 40.4 (B), 53.0 (A), 53.9 (B), 104.5 (B), 111.4 (B), 112.4 (B), 113.0 (A), 117.4 (A), 117.8 (B), 118.9 (B), 119.0 (B), 120.0 (A), 121.6 (B), 125.7 (B), 126.5 (B), 128.7 (B), 129.0 (A), 136.3 (B), 148.6 (B), 168.2 (B). − $C_{18}H_{19}ClN_4O$ (342.8): calcd. C 63.06, H 5.59, N 16.34; found C 63.22, H 5.86, N 16.13.

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