Synthesis and Spectroscopy of Novel-α-Pyrazolylglycine Derivatives

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The synthesis of α-pyrazolylglycine derivatives (**7a-d)** with different substituents, starting from glycine have been prepared. The spectroscopy of intermediate compounds and the final amino acids have been discussed.

INTRODUCTION

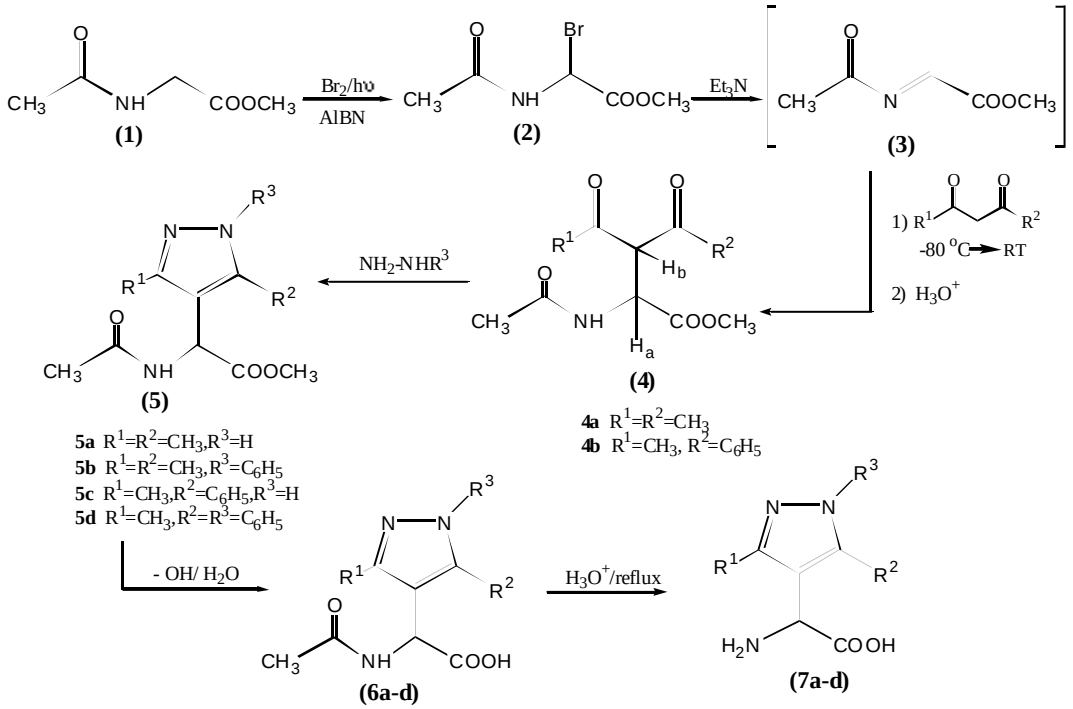
α-Amino acids serve a central role in biology and chemistry being the fundamental constituents of proteins and mediators of nitrogen metabolism and provide raw material for a large number of biologically important primary and secondary metabolites.[1] The number of naturally occurring α-amino acids has grown substantially from 20-amino acids normally found in proteins to over 700.[2] In addition there has been a tremendous surge of interest in preparation of relatively inaccessible unnatural amino acids whose potential biological properties and general synthetic utilities are just beginning to be realized. Of the methods presently available, those derived from electrophilic glycine equivalents are worthmentioning.[3]

In the course of our study on cyclization reactions[4-7] of different β-diketones with hydrazine derivatives, we have been able to prepare α-pyrazolyl-α-amino acids *via* Scheme I. In this paper we report the synthesis and spectroscopy of these amino acids and the intermediate compounds.

RESULTS AND DISCUSSION

The acetylation of glycine was carried out by a reported method[8] with the same yields and melting points of aceturic

Scheme I



acid. Esterification of aceturic acid[9] with $SOCl_2/CH_3OH$ gave (**1**) in good yield. Photolytic bromination of ester (**1**) with NBS/CCl_4 did not give satisfactory results; however, bromination with Br_2/CCl_4 in presence of AIBN (azobisisobutyronitrile), a radical initiator, resulted in the formation of α-bromo esters (**2**) in good yields.[10] On treatment of (**2**) with tertiary amine, highly reactive α-acyliminoacetate (**3**) was found *in situ* which on reaction with β-diketones gave (**4a-b**) in reasonably good yields. These 1,3-diketo derivatives, on cyclocondensation with hydrazine derivatives, afforded *N*-protected α-pyrazolylglycine esters (**5a-d**) which were hydrolysed to *N*-protected amino acids (**6a-d**). *N*-acetylated amino acids (**6a-d**) were subjected to enzymatic deacetylation with acylase which surprisingly failed. This can be attributed to steric hindrance due to the bulky nature of the substrate during the reaction with the enzyme.

Following Scheme I, the racemic α-amino acids (**7a-d**) could be prepared successfully by deacetylation of (**6a-d**) under acidic conditions.

In the ^{1}H-NMR spectra of compounds (**4a-b**), two methine protons (Ha and Hb) showed a doublet of doublet and a doublet at δ 5.36-5.8 and δ 4.45-6.3, respectively. In (**5a-d**) the methine protons showed coupling with the protons of N-H and showed a doublet in the region δ 5.28-6.4. In compound (**5b**), these methyl groups linked with a pyrazole ring showed separate singlets due to unequal environmental conditions.

Compounds (**6a-d**) obtained by saponification of the esters (**5a-d**) were identified only by IR spectra where the C=O vibrations were observed at 1720-1762 cm^{-1} in place of carbonyl stretching of ester group at 1704-1785 cm^{-1}. Moreover, C-O-C stretching of ester groups at 1105-1199 cm^{-1} were also absent in these compounds. The purity of acids were confirmed by elemental analysis. In the ^{1}H-NMR spectra of (**7a-d**), the methine proton appeared as singlet at δ 5.2. The mass spectra of (**7a-d**) showed base peaks due to the loss of –COOH group. The primary fragmentation consistent in all of the compounds was characterized by the presence of *m/z* $M^{+\bullet}$ -44] and $M^{+\bullet}$ -45] due to the loss of CO_2 and –COOH from the molecular ion, respectively.

EXPERIMENTAL

Chemicals

The following chemicals obtained from Sigma and Aldrich were used throughout this work without further purification namely, glycine, methyl sulphate, ammonia solution, acetone, alcohol, carbon tetrachloride, azobisisobutyronitrile, triethylamine, THF, citric acid, sodium hydrogen carbonate, ethyl acetate, sodium sulphate, petroleum ether, ethanol, hydrazine, phenylhydrazine, poatssium hydroxide, hydrochloric acid and magnesium sulphate. Ethanol was further purified by the reported method.[8]

PREPARATION OF COMPOUNDS

Methyl 2-acetylamino-4-oxo-3-(1′-oxoethyl/phenylmethyl)-pentanoates (4a-b)

The acylimino ester (**3**) was generated by addition of triethylamine (0.75 mL, 5.5 mmol) to bromo ester (**2**) in dry THF at -78 °C. After 30 minutes the β-dicarbonyl compound (6 mmol) stirred with triethylamine (6 mmol) in dry THF at room temperature for 30 minutes, was added slowly to the acylimino ester. After warming, the mixture was stirred for 12 hours at room temperature. Then aqueous 20% citiric acid (5 mL) was added and the solution was neutralized with aqueous sodium hydrogen carbonate. The product was extracted with ethyl acetate (3×), dried (Na_2SO_4) and evaporated *in vacuo*. Purification was possible by recrystallization from ethyl acetate/pet.ether or by column chromatography using ethyl acetate/pet.ether as eluent. **Methyl 2-acetylamino-4-oxo-3-(1′-oxoethyl)pentanoates (4a):** yield 44%, m.p. 103-5 °C. IR (ν_{max}, KBr, cm^{-1}): 3304, 2956, 1749, 1725, 1656, 1548. ^{1}H-NMR ($CDCl_3$, δ-values): 2.0 (s, 3H, $NCOCH_3$), 2.23 (s, 3H, $COCH_3$), 2.32 (s, 3H, $COCH_3$), 3.71 (s, 3H, $COOCH_3$), 4.46 (d, 1H Hb, J = 2 Hz), 5.26 (dd, 1H, Ha, J = 5 Hz). **Methyl 2-acetylamino-4-oxo-3-(1′-oxophenylmethyl)-pentanoates (4b):** yield 48%, m.p. 96-9 °C. IR (ν_{max}, KBr, cm^{-1}): 3280, 3076, 2956, 1758, 1722, 1650, 1602, 1584, 1527, 1446, 1329, 1284, ^{1}H-NMR ($CDCl_3$, δ-values): 2.03 (s, 3H, $COCH_3$), 2.36 (s, 3H, N-$COCH_3$), 3.68 (s, 3H, $COOCH_3$), 4.72 (s, 1H, Hb), 5.3 (dd, 1H, Ha, J = 7 Hz), 7.62 (m, 5H, Ar-H).

Methyl 2-acetylamino-2-(substituted pyrazol-4-yl)acetates (5a-d)

Compound (**4**) (5 mmole) dissolved in ethanol (50 mL) and after addition of hydrazine hydrate (100%) or phenylhydrazine (5 mmol), the mixture was refluxed for 2-3 hours. After evaporation to dryness the product was purified by recrystallization from ethyl acetate/pet.ether or by column chromatography. **Methyl 2-acetylamino-2-(3,5- dimethyl-pyrazol-4-yl)acetate (5a):** yield 61%, m.p. 95 °C. IR (ν_{max}, KBr, cm^{-1}): 3292, 1740, 1647, 1536, 1122. ^{1}H-NMR ($CDCl_3$, δ-values): 1.92 (s, 3H, CH_3CO), 2.16 (s, 6H, 2×CH_3-), 3.61 (s, 1H), 4.01 (s, 3H, $COOCH_3$), 5.28 (s, 1H), 7.22 (s, 1H). **Methyl 2-acetylamino-2-(3,5-dimethyl-1- phenylpyrazol-**

4-yl)acetate(5b): yield 70%, m.p. 140-1 °C. IR (ν_{max}, KBr, cm^{-1}): 3078, 2956, 2848, 1752, 1635, 1137. ^{1}H-NMR ($CDCl_3$, δ-val ues): 2.05 (s, 3H, -$COCH_3$), 2.28 (s, 3H, CH_3-), 3.33 (s, 3H, -CH_3), 3.75 (s, 3H, $COOCH_3$), 5.54 (d, 1H, Ha, *J* = 1.6 Hz), 7.34 (m, 5H, Ar-H). **Methyl 2-acetylamino-2-(3-methyl-5-phenylpyrazol-4-yl)ace tate(5c):**yield 54%. m.p. 182-3 °C. IR (ν_{max}, KBr, cm^{-1}): 3238, 3064, 2956, 1749, 1662, 1539, 1128. **Methyl 2-acetylamino-2-(3-methyl-1,5-diphenyl pyrazol-4-yl)ace tate(5d):**yield 58%, m.p. 172 °C. IR (ν_{max}, KBr, cm^{-1}): 3298, 3064, 3004, 2962, 1752, 1692, 1599, 1506, 1128.

2-Acetylamino-2-(substituted pyrazol-4-yl)acetic acids (6a-d)

The *N*-pro tected amino methyl es ters (**5a-d**) (1 mmol) were refluxed for one hour with a mix ture of eth a nol (20 mL) and 1N KOH (1.5 mL). Then the sol vent was evap o rated under re duced pres sure, the res i due dis solved in wa ter (20 mL), acid i fied with 1N HCl (2 mL) and ex tracted with ethyl ac e tate (3 × 20 mL). The or ganic layer was dried with an hy drous $MgSO_4$ and evap o rated to dry ness. The crude acid was pu rified by recrystallization from ethyl ac e tate/pet.ether. **2-Acetylamino-2-(3,5-dimethylpyrazol-4-yl)acetic acids (6a):** yield 51%, m.p. 203 °C. IR (ν_{max}, KBr, cm^{-1}): 3351, 3192, 2940, 1742, 1652, 1575, 1529, 1511, 1477, 1321, 1262, 1101, 1047, 721, 715. El e men tal Analysis: ($C_9H_{13}N_3O_3$; 211) Calc. (Found): C = 51.18 (51.02); H = 6.16 (5.95); N = 19.9 (19.7). **2-Acetylamino-2-(3,5-dimethyl-1-phenylpyrazol-4-yl)ace tic ac ids (6b):** yield 57%, m.p. 186.3 °C. IR (ν_{max}, KBr, cm^{-1}): 3941, 3313, 3237, 1729, 1629, 1535, 1432, 1401, 1387, 1271, 1120, 1034, 739. Elemental Analysis: ($C_{15}H_{17}N_3O_3$; 287) Calc. (Found): C = 62.7 (62.2); H = 5.9 (5.1); N = 14.6 (14.2). **2-Acetylamino-2-(3-methyl-5-phenyl pyrazol-4-yl)ace tic ac ids (6c):** yield 65%, m.p. 207 °C. IR (ν_{max}, KBr, cm^{-1}): 3208, 3051, 2955, 1762, 1631, 1540, 1448, 1382, 1290, 1260, 1209, 1123, 1070, 985, 700, 651. ElementalAnalysis: ($C_{14}H_{15}N_3O_3$; 273) Calc. (Found): C = 61.5 (60.98); H = 5.49 (5.11); N = 15.38 (14.89). **2-Acetylamino-2-(3-methyl-1,5-diphenylpyrazol-4-yl)ace tic ac ids (6d):** yield 61%, m.p. 192 °C. IR (ν_{max}, KBr, cm^{-1}): 3256, 3060, 3010, 2857, 1728, 1647, 1600, 1522, 1480, 1371, 1335, 1320, 1256, 1214, 1156, 1101, 1039, 760. Elemental Analysis: ($C_{20}H_{19}N_3O_3$; 349) Calc. (Found): C = 68.76 (68.14); H = 5.4 (5.05); N = 16.86 (16.33).

(Substituted pyrazol-4-yl)glycines (7a-d)

The sus pen sion of *N*-acetylamino acid (1 mmol) in 20 mL of 10% HCl was refluxed for 50 hours. A cold mix ture was ex tracted with ethyl ac e tate and aque ous phase was applied to an ion ex change col umn (Amberlite CG 120, 20 mL of resin bed). The col umn was eluted with fol lowed by 2% aque ous NH_3 so lu tion. The aq. am mo nia frac tion was evap orated *in vacuo*. The crude prod ucts were recrystallized from eth a nol/ac e tone. **(3,5-Dimethylpyrazol-4-yl)glycine) (7a):** yield 69%, m.p. 233 °C (decom.). IR (ν_{max}, KBr, cm^{-1}): 2908, 1641, 1518, 1446, 1377, 1344, 1287, 1212, 1149, 1044, 999, 894, 825, 738, 696. ^{1}H-NMR (D_2O, δ-val ues): 2.25 (s, 6H, 2 × CH_3-), 5.20 (s, 1H, Ha). MS: *m/z* (%): 169 ($M^{+\bullet}$, 1.15), 125 (17), 124 (100), 122 (34), 109 (26), 108 (37), 97 (38), 95 (7), 56 (17), 52 (10). **(3,5-Dimethyl-1-phenylpyrazol-4-yl)-glycine) (7b):** yield 72%, m.p. 220 °C (decom.). IR (ν_{max}, KBr, cm^{-1}): 3064, 1635, 1503, 1431, 1380, 1206, 1116, 1017, 693, 759. ^{1}H-NMR (D_2O, δ-val ues): 2.12 (s, 3H, -CH_3), 2.18 (s, 3H, -CH_3), 5.2 (s, 1H, CH), 7.4 (s, 5H, Ar-H). MS: *m/z* (%): 245 ($M^{+\bullet}$, 2%), 201 (21), 200 (100), 185 (35), 184 (40), 173 (16), 77 (59). **(3-Methyl-5-phenylpyrazol-4-yl)glycine (7c):** yield 58%, m.p. 230 °C (decom.). IR (ν_{max}, KBr, cm^{-1}): 3148, 2866, 1605, 1527, 1488, 1452, 1368, 1344, 1296, 1263, 1215, 1155, 1098, 999, 969, 792, 771, 729, 693. ^{1}H-NMR (D_2O, δ-value): 2.52 (s, 3H, -CH_3), 5.2 (s, 1H, CH), 7.2 (s, 5H, Ar-H). MS: *m/z* (%): 231 ($M^{+\bullet}$,1.5), 187 (26), 186 (100), 184 (13), 171 (23), 170 (14), 159 (15), 77 (32). **(3-Methyl-1,5-diphenylpyrazol-4-yl)glycine(7d):** yield 63%, m.p. 216 °C (decom.). IR (ν_{max}, KBr, cm^{-1}): 3364, 3160, 3046, 2716, 2548, 1617, 1506, 1449, 1431, 1371, 1323, 1296, 1262, 1107, 1074, 1011, 969, 912, 825, 792, 762, 696. ^{1}H-NMR (D_2O, δ-value): 2.24 (s, 3H, -CH_3), 4.07 (s, 1H, -CH), 7.25 (s, 10H, Ar-H). MS: *m/z* (%): 307 ($M^{+\bullet}$, 1.5), 263 (30), 262 (100), 246 (19), 247 (12), 131 (10), 77 (59).

INSTRUMENTATION

The melt ing points are un cor rected and re corded on a Gallenkamp dig i tal melt ing point ap pa ra tus. For re cord ing IR, ^{1}H-NMR and mass spec tra, a Hitachi 270-50, Jeol Model JNM FX 900 and Varian Mat CH-5 were used, re spec tively. The el e men tal anal y sis was car ried out on Carlo Erba Model DP 200. The NMR spec tra of all the com pounds were recorded in $CDCl_3$ ex cept the fi nal amino ac ids (**7a-d**), where D_2O was used as sol vent.

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Key Words

α-Amino acids; β-Diketones; Pyrazolylglycine derivatives.

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