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Susceptibility of Methyl 3-Amino-1H-pyrazole-5-carboxylate to Acylation

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Susceptibility of Methyl 3-Amino-1*H*-pyrazole-5-carboxylate to Acylation

Anna Kusakiewicz-Dawid, Łukasz Górecki, Elżbieta Masiukiewicz, and Barbara Rzeszotarska
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Abstract: In the search for a new method of synthesis of hybrid peptides with aminopyrazole carboxylic acid, we tried to force selective acylation at the aromatic amino group instead of at the ring nitrogen atom with fairly gentle acylating agents. The acylating agents used were acid anhydrides: acetic anhydride, *tert*-butyl pyrocarbonate, and 2-(2-methoxyethoxy)ethoxyacetic acid/dicyclohexylcarbodiimide. We succceded in acylation at this amino group with almost none at the ring nitrogen atom. Sometimes, however, acylation in small quantities at the ring nitrogen atom was observed as a by-product. To remove this by-product, imidazole was used. Thus, we were able to obtain the hybrid peptides in question with no protection and subsequent removal required. We synthesized a few these free peptides with no protection of the pyrazole ring. This is a simpler method than that being used currently.

Keywords: Acetylation, acylation, aromatic amine, 2-(2-methoxyethoxy) ethoxyacetylation, methyl 3-amino-1*H*-pyrazole-5-carboxylate, *tert*-butyloxycarbonylation

A series of pathological processes is associated with the formation of β-sheet structure and consecutive protein aggregation in the form of β-amyloid deposition.[1] One of the most promising approaches aims at the prevention of β-amyloid aggregation by using a specially designed β-sheet peptide ligand containing methyl 3-amino-1*H*-pyrazole-5-carboxylate,[1] a

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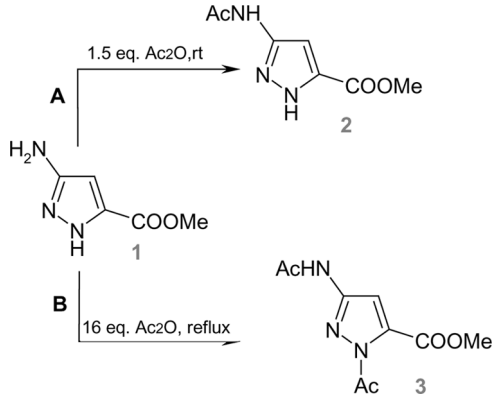
so-called β-sheet breaker. However, the synthesis of these peptides is difficult, because no amide coupling could be effected under any of the reaction conditions tested.[2] In all cases, the ring NH group has been found to be more reactive than the aromatic amine group, thus leading to ring acylation instead of peptide coupling.[2] Therefore, protection of the pyrazole nitrogen is first required.[1–3] This process is not univocal, as two nitrogen atoms are present in the pyrazole ring and two products are formed. It was assumed that the susceptibility of the nitrogen atom to acylation is dependent on the type and amount of acylating agent.

Therefore, methyl 3-amino-1*H*-pyrazole-5-carboxylate **1** acylation was treated with 1.5 equivalent of Ac_2O. In contrast to the previous statement,[2] the monoacylated product **2** was always obtained at the exoamino group (Scheme 1A). After 1 h of reaction with Ac_2O in dioxane at room temperature, there was 89% isolated product monoacetylated at the amino group. No acetylation at the ring nitrogen atom was observed. With 16 equivalents of acylation agent Ac_2O at reflux, after 4 h 46% isolated yield of diacylated product **3** was obtained (Scheme 1B).

For formation of the *tert*-butoxycarbonyl (Boc) derivative at the exoamino group, action of *tert*-butyl pyrocarbonate at a higher temperature, preferably in the presence of a tertiary amine, is required. It can be observed in the ^{1}H NMR spectrum that the signal of NH is strongly deshielded vs. tetramethylsilane (TMS, 12.3 ppm). This would suggest that NH is strongly susceptible to the formation of an anion in the presence of a tertiary amine. After 8 h of reaction with *tert*-butyl pyrocarbonate in dioxane, in the absence of a tertiary amine at reflux and with consecutive treatment with imidazole to remove the *tert*-butoxycarbonyl derivative at the ring nitrogen atom (Scheme 2C), **5** was obtained in



Scheme 1. Acetylation of **1**.

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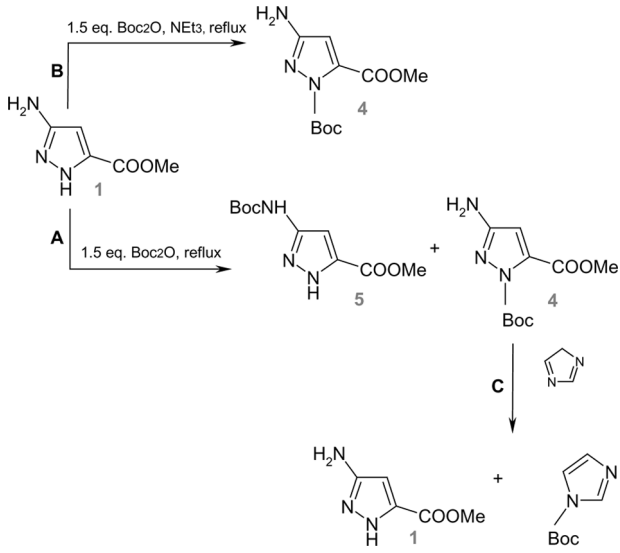
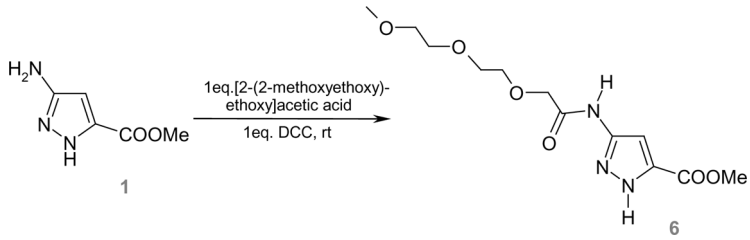


Scheme 2. *tert*-Butyloxycarbonylation of **1**.

analogy to the monoacetylated product in 72% isolated yield (Scheme 2A). In the presence of a tertiary amine with *tert*-butyl pyrocarbonate, **4** is formed; thus the ring nitrogen atom is acylated (Scheme 2B).

2-(2-Methoxyethoxy)ethoxyacetylation, which is used for the pronounced increase in solubility of product in water, follows the same course. Using dicyclohexylcarbodiimide in dioxane as a condensing agent at room temperature after 22 h, without ring nitrogen atom acylation, **6** was obtained in 75% yield (Scheme 3).

The obtained results show the possibility for the synthesis of the peptidic ligands in question and additionally without the protection of nitrogen in the pyrazole ring. Some peptide examples **7**–**9** were synthesized (Schemes 4 and 5). This method of synthesizing these compounds is simpler than that



Scheme 3. 2-(2-Methoxyethoxy)ethoxyacetylation of **1**.

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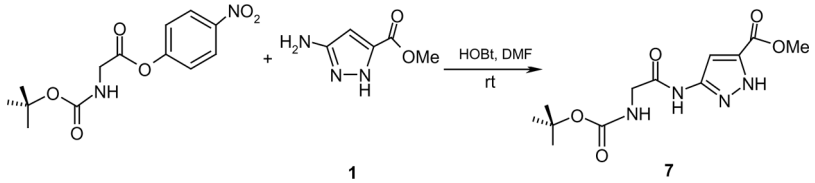


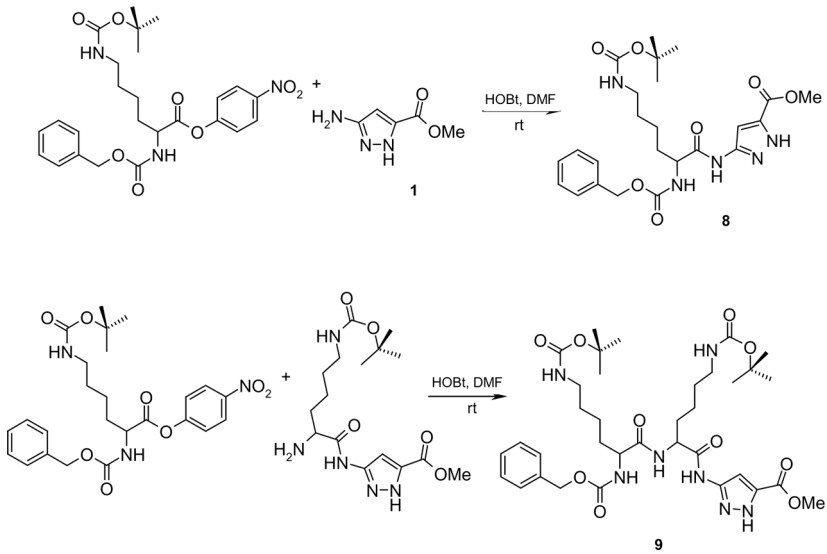
Scheme 4. Synthesis of methyl 3-(N^{α}-*tert*-butoxycarbonyl-glycylamino)-1*H*-pyrazole-5-carboxylate (**7**).

proposed by Schrader et al.[1–3] where protection of the pyrazole ring is required. The synthesis of such peptides without protection of the pyrazole ring is currently under way in this laboratory.

EXPERIMENTAL

General Procedure

The substrate methyl 3-amino-1*H*-pyrazole-5-carboxylate (**1**) of 97.9% purity (determined by high-performance liquid chromatography, HPLC)



Scheme 5. Synthesis of methyl 3-(N^{α}-benzyloxycarbonyl-N^{ε}-*tert*-butoxycarbonyl-L-lysylamino)-1*H*-pyrazole-5-carboxylate (**8**) and methyl 3-(N^{α}-benzyloxycarbonyl-N^{ε}-*tert*-butoxycarbonyl-L-lysyl-N^{ε}-*tert*-butoxycarbonyl-L-lysylamino)-1*H*-pyrazole-5-carboxylate (**9**).

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was obtained from commercial 3-nitro-1*H*-pyrazole-5-carboxylic acid of 99.6% purity (Aldrich) through the method highly recommended for esterification in peptide chemistry wherein methanol and an excess of thionyl chloride[4] (93% yield and 99.4% purity) are used. The methyl ester was produced in its free form without hydrochloride (through elemental analysis and mass spectroscopy). Consecutive reduction of the nitro group with hydrogen in the presence of Pd/C[5] gave 81% yield of amine of 99.7% purity.

The solvents from the reaction mixtures were removed in vacuo on a rotary evaporator at a bath temperature not exceeding 40°C. Thin-layer chromatography (TLC) was performed on silica gel (DC Alufolien Kieselgel Merck 1.05553) with detection by means of chlorine–KI–tolidine in the solvent systems (A) $CHCl_3$/MeOH/AcOH (90:8:2), (B) $CHCl_3$/MeOH/AcOH (95:5:3), (C) n-BuOH/AcOH/AcOEt/H_2O (1:1:1:1), (D) $CHCl_3$/acetone (9:1), (E) n-BuOH/AcOH/H_2O (4:1:1), and (F) C_6H_6/MeOH/acetone/pyridine/AcOH (24:4:2:2:1). Purities for the compounds were checked by HPLC by a Beckman "System Gold" with an Alltech Alltime C18 (RP 5 μm, 150 × 4.6 mm) column and a loop (5 μl). The mobile phases were 0.1% TFA/acetonitrile (A) 90:10, (B) 70:30, (C) 50:50, and (D) 40:60 at a 1 ml min^{-1} rate of flow. Detection was made at 210 nm. Melting points were recorded on a DSC-2010 calorimeter (Thermal Analysis Instruments) under nitrogen in a closed copper vessel with a heating rate of 2°C min^{-1}. NMR spectra were measured on a Brucker Avance 400-MHz spectrometer in dimethyl sulfoxide (DMSO-d_6) or $CDCl_3$ in the presence of tetramethylsilane. Resonances were based on heteronuclear multiple bond correlation (HMBC) and heteronuclear single quantum correlation (HSQC) spectra. Fourier transform infrared (FTIR) spectra were recorded on a PU 9800 Philips analytical spectrometer at 2 cm^{-1} resolution in KBr or $CHCl_3$.

Methyl 3-Nitro-1*H*-pyrazole-5-carboxylate

Absolute methanol (5.6 ml, 138 mmol) was cooled to −15°C and thionyl chloride (0.8 ml, 11 mmol) was added dropwise under stirring. After 15 min, the solution was followed by 3-nitro-1*H*-pyrazole-5-carboxylic acid (1.26 g, 8 mmol). Stirring continued at −15°C for 15 min and then at room temperature for 24 h. The volatiles were evaporated, and the resulting colorless crude precipitate was washed with acetone to neutralize the pH and decay the sulfur compounds. It was then crystallized with diethyl ether and hexane. A colorless solid resulted (1.28 g, 93% yield), mp 145.5°C, mp lit.[2] 138°C; TLC: R_f (A) 0.74; HPLC (B): t_R

5.29 min, 99.4% purity. Analysis calcd. for $C_5H_5N_3O_4$: C, 35.09%; H, 2.95%; N, 24.56%. Found: C, 35.18%; H,3.04%; N, 24.47%.

Methyl 3-Amino-1*H*-pyrazole-5-carboxylate (1)

Methyl 3-nitro-1*H*-pyrazole-5-carboxylate was suspended in methanol (40 ml), 10% Pd/C (0.26 g) was added, and a Parr apparatus was filled with hydrogen at five bars pressure. After 24 h, the catalyst was filtered off and washed with methanol. The methanol was evaporated, and the residue was crystallized with ethanol (10 ml). A colorless powder resulted (1.14 g, 81% yield), mp 144.3°C; TLC: R_f (A) 0.43; R_f (C) 0.83; HPLC (A): t_R 2.78 min, 99.7% purity. Analysis calcd. for $C_5H_7N_3O_2$; C, 42.55%; H, 4.96%; N, 29.79%. Found: C, 42.70%; H, 5.00%; N, 29.71%. ^{1}H NMR (DMSO-d_6) δ (ppm): 3.742 (s, 3H, OCH_3), 5.042 (s, 2H, NH_2), 5.763 (s, 1H, C^4H), 12.253 (s, 1H, NH_{ring}).

Methyl 3-(Acetylamino)-1*H*-pyrazole-5-carboxylate (2)

Ester **1** (0.16 g, 1.09 mmol) was suspended in dioxane (2 ml), and Ac_2O (0.15 ml, 1.5 mmol) was added. After several minutes, a precipitate appeared, which after a further hour was filtered off to give **2** (0.177 g, 89% yield), mp 220°C, TLC (A): R_f 0.57; HPLC (A): t_R 6.99 min, 98.3% purity. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.017 (s, 3H, CH_3CO), 3.828 (s, 3H, OCH_3), 6.989 (1H, C^4H), 10.622 (s, 1H, NH_{amide}), 13.556 (s, 1H, NH_{ring}); ^{13}C NMR (DMSO-d_6) δ (ppm): 22.95 ($CH_{3acetyl}$), 52.02 ($CH_{3\text{-}ester}$), 99.36 (C^4), 132.65 (C^3), 148.00 (C^5), 159.27 ($C{=}O_{ester}$), 167.84 ($C{=}O_{amide}$). FTIR (KBr) (cm^{-1}) 3313, 3181 (νNH), 1726 (ν $C{=}O_{amide}$), 1694 (ν $C{=}O_{ester}$). Analysis calcd. for $C_7H_9N_3O_3$: C, 45.89%; H, 4.95%; N, 22.93%. Found: C, 46.05%; H, 5.07%; N 23.05.

Methyl 3-(Acetylamino)-1-acetyl-1*H*-pyrazole-5-carboxylate (3)

Ester (**1**) (0.14 g, 1.0 mmol) was suspended in Ac_2O (1.52 ml, 16 mmol), heated for 4 h, and left standing overnight. The formed precipitate was filtered off (0.10 g, 46% yield), mp 153°C, TLC (A): R_f 0.87; HPLC (B): t_R 3.7 min, 98.2% purity. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.192 (s, 3H, $CH_{3acetyl\ amide}$), 2.679 (s, 3H, $CH_{3acetyl\ ring}$), 3.861 (s, 3H, CH_{3ester}), 7.019 (s, 1H, C^4H), 10.342 (s, 1H NH_{amide}); ^{13}C NMR (DMSO-d_6) δ (ppm): 23.40 ($2 \times CH_{3acetyl}$), 52.32 (CH_{3ester}), 98.55 (C^4), 141.11 (C^3),

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145.10 (C_5), 161.33 ($C{=}O_{ester}$), 167.75 ($C{=}O_{amide}$), 173.72 ($C{=}O_{ring}$); FTIR (cm^{-1}) (KBr): 3350 (νNH), 1741, 1725, 1709 ($\nu C{=}O_{amide}$, $\nu C{=}O_{ester}$); ($CHCl_3$): 3358 (νNH), 1730 ($\nu C{=}O_{amide}$ and $C{=}O_{ester}$). Analysis calcd. for $C_9H_{11}N_3O_4$: C, 48.00%; H, 4.92%; N, 18.66%. Found: C, 48.08%; H, 4.80%; N, 18.48%.

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Methyl 3-(*tert*-Butoxycarbonylamino)-1*H*-pyrazole-5-carboxylate (5)

Ester (**1**) (0.282 g, 2 mmol) was dissolved in dioxane (8 ml), *tert*-butyl pyrocarbonate (0.654 g, 3 mmol) was added, and the entire mixture was then refluxed for 8 h. The solution was left standing overnight. Imidazole (0.613 g, 9 mmol) was added and refluxed for 2 h, and dioxane was evaporated. The residue was dissolved in ethyl acetate, extracted with HCl (0.5 M, 3 × 5 ml), washed with water, and dried, and the ethyl acetate was evaporated. A colorless powder resulted (0.349 g, 72% yield), mp 179–182°C; TLC: (B) R_f 0.70; HPLC: (C) t_R 3.1 min, 99% purity; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.450 (s, 9H, $CH_{3urethane}$), 3.827 (s, 3H CH_{3ester}), 6.765 (s, 1H, C^4H), 9.822 (s, 1H, NH_{amide}), 13.431 (s, 1H, NH_{ring}); ^{13}C NMR (DMSO-d_6) δ (ppm): 27.84 ($CH_{3urethane}$), 51.87 ($CH_{3\text{-}ester}$), 79.05 ($C_{quaternary}$), 98.46 (C^4), 132.53 (C^3), 148.16 (C^5), 152.67 ($C{=}O_{amide}$), 159.22 ($C{=}O_{ester}$); FTIR (cm^{-1}) (KBr): 3326, 3181 (νNH), 1736, 1720 (νC=O); ($CHCl_3$): 3441, 3329 (νNH), 1719 (νC=O). Analysis for $C_{10}H_{15}N_3O_4$ calcd.: C, 49.79%; H, 6.27%; N, 17.42%. Found: C, 49.63%; H, 6.16; N, 17.53.

Methyl 1-*tert*-Butoxycarbonyl-3-amino-1*H*-pyrazole-5-carboxylate (4)

Ester (**1**) (0.141 g, 1 mmol) was dissolved in dioxane (6 ml), triethylamine (0.104 ml, 1.5 mmol) and *tert*-butyl pyrocarbonate (0.327 g, 1.5 mmol) were added, and the whole mixture was heated at reflux for 1 h. The solvent was evaporated, and the residue was dissolved in ethyl acetate, extracted with 5% citric acid (2 × 5 ml), washed with water, and dried. Ethyl acetate was then evaporated. The precipitate was treated with diethyl ether and filtered off. A colorless powder resulted (1.08 g, 45% yield), mp 135–137°C; TLC: (B) 0.65; HPLC: (C) t_R 3.47 min, 98% purity; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.591 (s, 9H, $CH_{3urethane}$), 3.803 (s, 3H, CH_{3ester}), 5.702 (s, 1H, C^4H), 6.535 (s, 2H, NH_2); ^{13}C NMR (DMSO-d_6) δ (ppm): 27.53 ($CH_{3urethane}$), 51.88 (CH_{3ester}), 81.47 ($C_{quaternary}$), 88.00 (C^4), 145.14 (C^5), 149.59 ($C{=}O_{amide}$), 152.11 (C^3), 162.17 ($C{=}O_{ester}$). FTIR (cm^{-1}) (KBr): 3487, 3353 (νNH_2), 1739 ($\nu C{=}O_{urethane}$), 1730 ($\nu C{=}O_{ester}$), 1609 (δNH_2); ($CHCl_3$): 3508, 3393 (νNH_2), 1737

($\nu C{=}O_{urethane}$), 1727 ($\nu C{=}O_{ester}$), 1613 (δNH_2). Analysis for $C_{10}H_{15}N_3O_4 \times 0.1$ *t*BuOH calcd.: C, 50.19%; H, 6.43%; N, 16.89%. Found: C, 49.79%; H, 6.37%; N, 16.55%.

Methyl 2-(2-Methoxyethoxy)ethoxyacetylamino-1*H*-pyrazole-5-carboxylate (6)

Ester (**1**) (0.705 g, 5 mmol) was dissolved in warm dioxane (12.5 ml). After cooling to room temperature, 2-(2-methoxyethoxy)ethoxyacetic acid (0.77 ml, 5 mmol) and dicyclohexylcarbodiimide (1.03 g, 5 mmol) were added, and after 40 h, dicyclohexylurea was filtered off and washed with dioxane (2×12.5 ml). The dioxane was then evaporated. The residue was dissolved in chloroform (5 ml), applied on a silica-gel column (60H Merck 1.05553, ϕ 4 cm, h 6 cm), and equilibrated with chloroform. The column was eluted with chloroform (50 ml) and followed with solutions with increased concentrations in acetone, from 2.5% to 32.5%. A colorless powder resulted (1.125 g, 75% yield), mp 82.5–83.5°C (83.86°C according to DSC); TLC: R_f (D) 0.49, (E) 0.57, (F) 0.66; HPLC: (B) t_R 2.71 min, 100% purity; ^{1}H NMR ($CDCl_3$) δ (ppm): 3.398 (s, 3H, $CH_{3glycol}$), 3.617–3.639 (m, 2H, $CH_{2glycol}$), 3.713–3.373 (m, 4H, $2 \times CH_{2glycol}$), 3.786–3.793 (m, 2H, $CH_{2glycol}$), 3.922 (s, 3H, OCH_3), 4.190 (s, 2H, $CH_{2glycol}$), 7.110 (s, 1H, C^4H), 9.845 (s, 1H, NH_{amide}), 11.263 (s, 1H, NH_{ring}); ^{13}C NMR ($CDCl_3$), δ (ppm): 52.20 (OCH_3), 58.88 ($CH_{3glycol}$), 70.07 ($CH_{2glycol}$), 70.30 ($CH_{2glycol}$), 70.44 ($CH_{2glycol}$), 71.21 ($CH_{2glycol}$), 71.64 ($CH_{2glycol}$), 98.54 (C^4), 137.67 (C^5), 143.87 (C^3), 161.13 ($C{=}O_{ester}$), 168.48 ($C{=}O_{amide}$). FTIR (cm^{-1}) (KBr): 3444, 3268, 3139 (νNH_{amide}, νNH_{ring}), 2989, 2959, 2928, 2882, 2848 (νCH_2), 1732 ($\nu C{=}O_{ester}$), 1653 ($\nu C{=}O_{amide}$), 1594 ($\nu C{=}N_{ring}$), (CH_2Cl_2): 3429, 3399, 3311 (νNH_{amide}, νNH_{ring}) 2916 (νCH_2) 1725 ($\nu C{=}O_{ester}$), 1696 ($\nu C{=}O_{amide}$), 1604 ($\nu C{=}N_{ring}$). Analysis for $C_{12}H_{19}N_3O_6$ calcd.: C, 47.84%; H, 6.36%; N, 13.95%. Found: C, 47.74%; H, 6.40%; N, 13.95%.

Methyl 3-(N^{α}-*tert*-Butoxycarbonyl-glycylamino)-1*H*-pyrazole-5-carboxylate (7)

Compound **1** (0.141 g, 1 mmol) was dissolved in DMF (2.3 ml), and Boc-Gly-ONp (0.355 g, 1.2 mmol) and HOBt $\times$ H_2O (0.153 g, 1 mmol) were added. After 21 h at room temperature, the solvent was removed. The residue was triturated with 5% $NaHCO_3$ (1.5 ml). The precipitate was filtered off and washed with 5% $NaHCO_3$ (3×1.5 ml), with water

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(2 × 2 ml), and with diethyl ether (2 × 2 ml). Compound **7** was obtained (0.221 g, 74% yield), mp 201.23°C; TLC (B): R_f 0.27; HPLC (C): $t_R = 2.16$ min, 100% purity; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.392 (s, 9H, $CH_{3\text{-}tBu}$), 3.718 and 3.733 (d, 2H, $CH_{2\text{-Gly}}$), 3.834 (s, 3H, OCH_3), 6.920 (s, lH, CH_{ring}), 7.067 (s, lH, $NH_{urethane}$), 10.645 (s, 1H, NH_{amide}), 13.563 (s, lH, NH_{ring}); ^{13}C NMR (DMSO-d_6) δ (ppm): 28.23 (3C, $CH_{3\text{-}tBu}$), 43.23 ($CH_{2\text{-Gly}}$), 51.99 (OCH_3), 78.11 ($C_{\text{-}tBu}$), 98.79 (C^4), 133.80 (C^5), 147.00 (C^3), 155.94 ($C{=}O_{urethane}$), 159.71 ($C{=}O_{ester}$), 167.87 ($C{=}O_{amide}$). Analysis calcd. for $C_{12}H_{18}N_4O_5$: C, 48.32%; H, 6.08%; N, 18.78%. Found: C, 48.56%; H, 6.19%; N, 18.98%.

Methyl 3-(N^{α}-Benzyloxycarbonyl-N^{ε}-*tert*-butoxycarbonyl-L-lysylamino)-1*H*-pyrazole-5-carboxylate (8)

Compound **1** (0.282 g, 2 mmol) was dissolved in DMF (3 ml), and Z-Lys(Boc)-ONp (1.103 g, 2.2 mmol) and HOBt × H_2O (0.306 g, 2 mmol) were added. After 24 h at room temperature, the solvent was removed. The residue (oil) was dissolved in ethyl acetate (12 ml) and extracted with 5% $NaHCO_3$ (4 × 3 ml), brine (3 × 3 ml), and water (2 × 3 ml). The ethyl acetate layer was dried with $MgSO_4$. The ethyl acetate was evaporated, and the residue (oil) was dissolved in chloroform (2.5 ml), applied on a column (60H Merck 1.05553, Φ 3 cm, h 6 cm), and equilibrated with chloroform (100 ml) and 2.5%, 5.0%, 7.5%, 10.0%, 15.0%, and 20.0% acetone in chloroform (each 50 ml) and 25% acetone in chloroform (150 ml). The solutions was evaporated; the precipitate was dissolved in ethyl acetate and extracted with 0.5 N HCl (2 × 2 ml) and brine (5 × 3 ml). The ethyl acetate layer was dried with $MgSO_4$. Compound **9** (0.532 g, 53% yield) was obtained, mp 59.96°C; TLC (B): R_f 0.51; HPLC: (D), t_R 3.16 min, 100% purity; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.403 (s, 9H, $CH_{3\text{-}t\text{Bu}}$), 1.492 (brs, 2H, $CH_{2\text{-Lys}}$), 1.797 (brs, lH, $CH_{2\text{-Lys}}$), 1.915 (brs, lH, $CH_{2\text{-Lys}}$), 3.076 (brs, 3H, $CH_{2\text{-Lys}}$), 3.921 (s, 4H, OCH_3), 4.527 and 4.541 (d, lH, α-CH), 4.730 (s, lH, $NH_{urethane\ Boc}$), 5.078–5.162 (m, 3H, $CH_{2\text{-}Z}$), 6.022 and 6.038 (d, lH, $NH_{urethane\ Z}$), 7.303 (s, lH, CH_{ring}), 7.326 (m, 5H, $CH_{arom.}$), 10.728 (s, lH, NH_{amide}); ^{13}C NMR (DMSO-d_6) δ (ppm): 22.62 ($CH_{2\text{-Lys}}$), 28.39 (3C, $CH_{3\text{-}t\text{Bu}}$), 29.68 ($CH_{2\text{-Lys}}$), 32.70 ($CH_{2\text{-Lys}}$), 40.12 ($CH_{2\text{-Lys}}$), 52.67 (OCH_3), 55.18 (α-CH), 67.25 ($CH_{2\text{-}Z}$), 79.27 ($C_{\text{-tBu}}$), 100.29 (C^4), 128.16–128.51 (5C, C^2-$C^6_{arom.}$), 134.59 (C^3), 136.05 ($C^1_{arom.}$), 147.07 (C^3), 156.19 ($C{=}O_{urethane\ Boc}$), 156.47 ($C{=}O_{urethane\ Z}$), 160.79 ($C{=}O_{ester}$), 170.16 ($C{=}O_{amide}$). Analysis calcd. for $C_{24}H_{33}N_3O_7$: C, 57.25%; H, 6.61%; N, 13.91%. Found: C, 57.12%; H, 6.37%; N, 13.88%.

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Methyl 3-(N^{α}-benzyloxycarbonyl-N^{ε}-*tert*-butoxycarbonyl-L-lysyl-N^{ε}-*tert*-butoxycarbonyl-L-lysylamino)-1*H*-pyrazole-5-carboxylate (9)

Compound **8** (0.532 g, 1.05 mmol) was dissolved in MeOH (7 ml); 10% Pd/C (0.053 g) and H_2 were added. After 3 h, the catalyst was filtered off and MeOH was evaporated. Lys(Boc)-Pz-OMe (0.306 g, 0.8 mmol) was obtained and dissolved in DMF (3 ml). Z-Lys(Boc)-ONp (0.441 g, 0.88 mmol) and HOBt × H_2O (0.135 g, 0.88 mmol) were added. After 20 h of stirring at room temperature, the DMF was evaporated. The residue was dissolved in chloroform (20 ml), extracted with 5% $NaHCO_3$ (4 × 2 ml), brine (4 × 4 ml), and water (2 × 4 ml), and dried with $MgSO_4$. Chloroform was evaporated, and ethyl ether was added to the residue and left standing in the refrigerator. The precipitate was filtered off (0.521 g), washed with ethyl ether (3 × 3 ml), and dissolved in warm methanol (8 ml). After cooling to room temperature, ethyl ether (20 ml) was added. Compound **9** (0.479 g, 62% yield, two crops) was obtained, mp 163.39°C; TLC (B): R_f 0.57; HPLC: (D) t_R 4.56 min, first crop 100% purity, second crop 98.8%. ^{1}H NMR (DMSO-d_6) δ (ppm): 1.365 (s, 22H, $CH_{3\text{-}t\text{Bu}}$ × 2, $CH_{2\text{-Lys}}$ × 2), 1.516 (s, 4H, $CH_{2\text{-Lys}}$ × 2), 1,610 (s, 4H, $CH_{2\text{-Lys}}$ × 2), 2.890 (s, 4H, $CH_{2\text{-Lys}}$ × 2), 3.836 (s, 3H, OCH_3), 4.014 (s, 1H, α-$CH_{\text{-Lys}}$), 1.417 (s, 1H, α-$CH_{\text{-Lys}}$), 5.041 (s, 2H, $CH_{2\text{-Z}}$), 6.771 (s, 2H, $NH_{\text{urethane Boc}}$ × 2), 6.996 (s, 1H, C^4), 7.324 (s, 1H, $NH_{\text{urethane Z}}$), 7.365 (m, 5H, $CH_{\text{arom.}}$), 8.037 (s, lH, $NH_{\text{amide Lys-Lys}}$), 10.745 (s, lH, $NH_{\text{amide Lys-pyrazole}}$), 13.597 (s, 1H, NH_{ring}); ^{13}C NMR (DMSO-d_6) δ (ppm): 22.58 ($CH_{2\text{-Lys}}$), 22.82 ($CH_{2\text{-Lys}}$), 28.18 (6C, $CH_{3\text{-}t\text{Bu}}$), 29.13 (2C, $CH_{2\text{-Lys}}$), 31.51 ($CH_{2\text{-Lys}}$), 31.69 ($CH_{2\text{-Lys}}$), 40.04 (2C, $CH_{2\text{-Lys}}$), 51.87 (OCH_3), 52.62 (α-$CH_{\text{-Lys}}$), 54.57 (α-$CH_{\text{-Lys}}$), 65.32 (CH_2-Z), 77.25 ($C_{\text{-}t\text{Bu}}$), 99,59 (C^4), 127.63 and 128.26 (5C, C^2-$C^6_{\text{arom.}}$), 132.99 (C^5), 136.97 ($C^1_{\text{arom.}}$), 144.50 (C^3), 155.49 (2C, $C{=}O_{\text{urethane Boc}}$), 155.91 ($C{=}O_{\text{urethane Z}}$), 159.47 ($C{=}O_{\text{ester}}$), 170.14 ($C{=}O_{\text{amide Lys-Lys}}$), 171.99 ($C{=}O_{\text{amide Lys-pyrazole}}$). Analysis calcd. for $C_{35}H_{53}N_7O_{10}$: C, 57.44%; H, 7.30%; N, 13.40%. Found: C, 57.07%; H, 7.31%; N, 13.47%.

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