HETEROCYCLES, Vol. 91, No. 12, 2015, pp. 2315 - 2326. © 2015 The Japan Institute of Heterocyclic Chemistry
Received, 2nd October, 2015, Accepted, 5th November, 2015, Published online, 27th November, 2015
DOI: 10.3987/COM-15-13333

SYNTHESIS OF NOVEL (*S*)-*N*-BOC-3-PYRAZOLYLALANINE-DERIVED *α*-AMINO ACIDS

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Abstract – Di-*tert*-butyl (*S*,*E*)-4-[(dimethylamino)methylidene]-5-oxopyrrolidine-1,2-dicarboxylate (**1**), available in three steps from L-pyroglutamic acid, was used as the starting material for the preparation of a small library of *N*-Boc-protected 3-pyrazolylalanine *tert*-butyl esters **3** using 'ring-switching' strategy with different hydrazine derivatives **2**. Acid-catalyzed deprotection (TFA/CH_2Cl_2) of **3** yielded free amino acids **4** isolated as dihydrochloride salts, while selective deprotection with HCl/EtOAc furnished *N*-Boc deprotected *tert*-butyl esters dihydrochlorides **5**. Hydrolysis under basic conditions furnished *N*-Boc-protected amino acid **6**.

INTRODUCTION

2-Substituted alkyl 3-(dimethylamino)prop-2-enoates and related enaminones are versatile reagents in heterocyclic synthesis, which have been extensively used in the synthesis of a broad spectrum of heterocyclic systems.[1] Some of the recent applications of enaminones extend to the preparation of functionalized heterocyclic compounds including natural product analogues[2,3] and to combinatorial synthesis of heterocycles.[4]

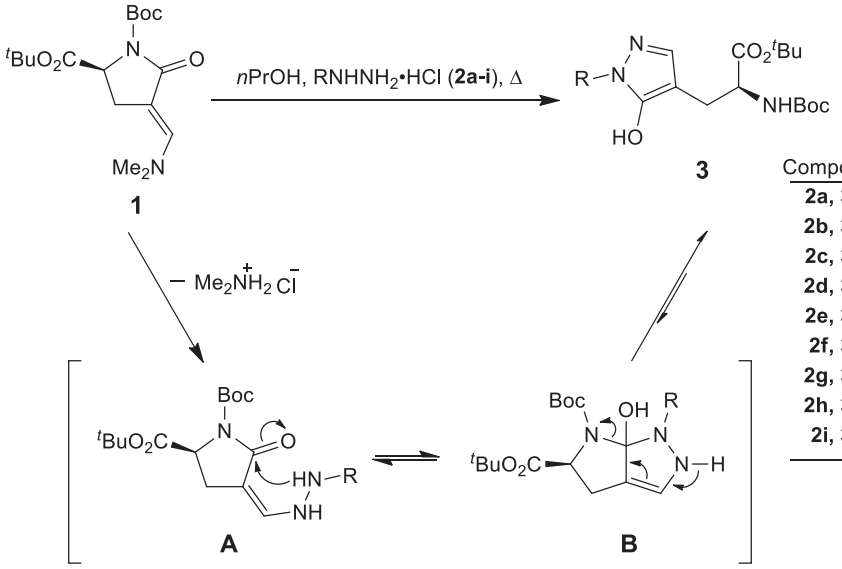
(*S*)-Pyroglutamic acid derived enaminones have been used as the key intermediates in the 'ring switching' processes towards different types of various non-proteinogenic *α*-amino acids, such as 3-heteroarylalanines bearing the pyrazole,[5-7] pyrimidine,[6,8] quinolizine, and 2*H*-2-pyranone residue,[9] (2*S*,4*R*)-[5,5,5-$^2H_3$]-leucine,[10,11] naturally occurring 4-alkylideneglutamic acids, 4-alkylglutamates, and 4-alkylprolines,[12,13] (2*S*,4*S*)-fluoroleucine[14] and (2*S*,4*S*)- and (2*S*,4*R*)-[5,5-$^2H_2$]-5,5-dihydroxyleucine,[15,16] 1,2-dithiolane analogues of leucine,[17] 4-heteroarylmethylideneglutamate analogues,[18] and 2-imino-3-methylenepyrrolidine derivative bactericides for *Erwinia amylovora* control.[19,20] They have also been used for the combinatorial solution-phase synthesis of (2*S*,4*S*)-4-acylamino-5-oxopyrrolidine-2-

carboxamides,[21] and for parallel solution-phase synthesis of (2*S*,4*E*)-4-(arylaminomethylidene)-pyroglutamic acids.[22]

Our previous studies in this field were performed with methyl (*S*,*E*)-1-benzoyl-4-[(dimethylamino)methylidene]-5-oxopyrrolidine-2-carboxylate to furnish the *N*-benzoylated 3-heteroarylalanine esters[7,9] as precursors for the corresponding free amino acids. Nevertheless, it is selective deprotection of the amino and the carboxy function that makes incorporation of a non-proteinogenic amino acid into an oligopeptide much easier. Therefore, our initial study was extended on application of easily available di-*tert*-butyl (*S*,*E*)-4-[(dimethylamino)methylidene]-5-oxopyrrolidine-1,2-dicarboxylate (**1**)[21] for the preparation of (*S*)-*N*-Boc-3-heteroarylalanine *tert*-butyl esters that can be, either deprotected completely to give the free amino acids, or deprotected selectively at the amino or the carboxy function. Herein, we report the preparation of novel Boc-protected 3-pyrazolylalanine-derived α-amino acids **3** from *N*-Boc-protected (*S*)-pyroglutamic acid derived enaminone **1** via 'ring switching' methodology using various hydrazine derivatives **2**. The possibility to easily fully or selectively deprotect amino acid derivatives **3** is also demonstrated.

RESULTS AND DISCUSSION

Enaminone **1** was prepared in three steps from commercially available (*S*)-pyroglutamic acid following the literature procedure.[21] 'Ring-switching' reactions of **1** with hydrazines **2a-i** required both, the elevated temperature and the acid catalyst. As shown previously, 'ring-switching' reactions of **1** with different dinucleophiles in acetic acid at elevated temperature (>90 °C) caused decomposition of the enaminone **1**,[7] while the same reactions applied on the Bz-protected methyl ester analogue of **1** proceeded according to the expectations, thus giving the corresponding 'ring-switched' products.[7,9] Following successful related example in the *N*-Boc-2-pyrrolidinone series,[23] reactions of **1** were performed under less acidic conditions, *i.e.* in boiling ^{n}PrOH with one equivalent of hydrazine **2** and in the presence of one equivalent of HCl (for details see the *Experimental part*). The corresponding products, alanine derivatives **3a-i**, were obtained in 28-92% yields. The 'ring-switching' reaction pathway is explainable by a two-step mechanism. First, the acid-catalyzed substitution of the dimethylamino group takes place with the more reactive primary amino group of the hydrazine derivative giving the intermediate **A**. In the case of (hetero)arylhydrazines **2b-h**, the primary amino group is more nucleophilic and less sterically hindered, whereas in the case of *tert*-butylhydrazine (**2i**), the higher reactivity of the primary amino group is due to steric hindrance of the more nucleophilic secondary amino group. Addition of the secondary nitrogen atom to the Boc-activated lactam carbonyl group of **A** gives the bicyclic intermediate **B**, which then tautomerizes via opening of the pyrrolidine ring, resulting in the regioselective formation of the final products **3a-i**.[1,7,9] As clearly shown previously, the resulting pyrazole ring adopts the 5-hydroxy tautomeric form (Scheme 1).[5,7,9]

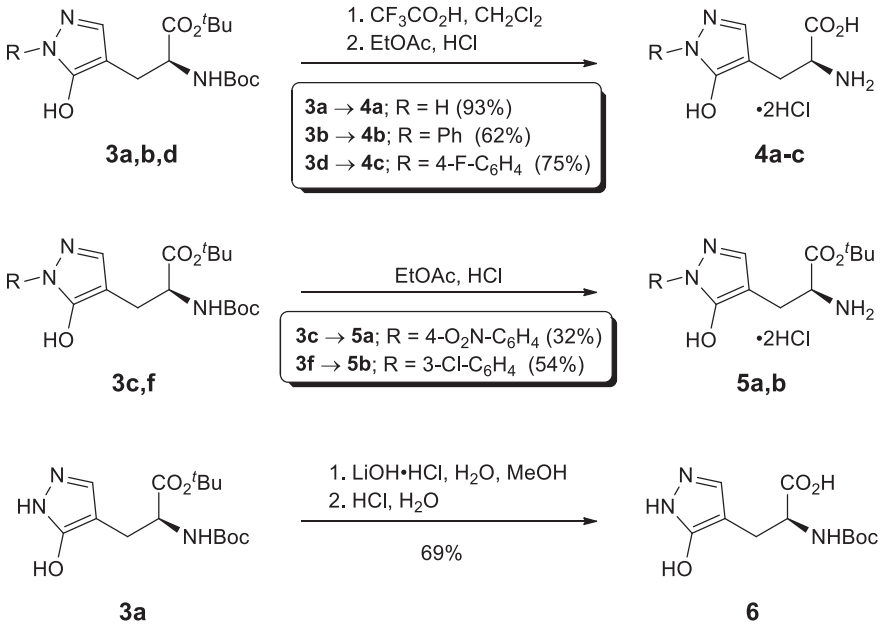


Compound	R	Yield (%)
2a, 3a	H	92
2b, 3b	Ph	45
2c, 3c	4-O_2N-C_6H_4	57
2d, 3d	4-F-C_6H_4	91
2e, 3e	4-Cl-C_6H_4	63
2f, 3f	3-Cl-C_6H_4	60
2g, 3g	2-Me-C_6H_4	57
2h, 3h	pyrimidin-2-yl	28
2i, 3i	^{t}Bu	37

Scheme 1

Next, attempts have been made to selectively deprotect, either the ^{t}Bu-ester, or the *N*-Boc group of **3**, or both. The global deprotection of **3a**,**b**,**d** was achieved with CF_3CO_2H in CH_2Cl_2. After the removal of volatile components, the oily residue was exposed to excess 2M HCl solution in EtOAc, followed by vigorous stirring, till filterable precipitate was formed. The so formed free amino acids **4a-c** were isolated as dihydrochlorides in 62-93% yields. The collected salts **4a-c** turned out to be hygroscopic and were, after drying in high vacuum, stored under argon. On the other hand, following the procedure by Rapoport and co-workers,[24] treatment of **3c** and **3f** in with a solution of HCl in EtOAc at room temperature furnished the corresponding *N*-Boc-deprotected *tert*-Bu-esters dihydrochlorides **5a** and **5b** in 32% and 54% yield, respectively, again as filterable hygroscopic precipitates. Simple alkaline workup of ester **3a** with LiOH in a mixture of H_2O and MeOH gave, after neutralization/extraction protocol, the *N*-Boc-protected amino acid **6** in 69% yield (Scheme 2).

The structures of novel compounds **3a-i**, **4a-c**, **5a**,**b**, and **6** were determined by spectroscopic methods (^{1}H-NMR, ^{13}C-NMR, 2D-NMR, IR, HRMS) and by elemental analyses for C, H, and N. Compounds **3a-f**,**i** were obtained in analytically pure form. Compounds **3g**,**h**, **4a-c**, **5a**,**b**, and **6** were not obtained in analytically pure form; their identities were confirmed by ^{13}C-NMR and HRMS. For compounds **3b-f** and **5a**, even after prolonged measurement, 1 or 2 of the non-equivalent carbon signals are missing in the respective ^{13}C-NMR spectra.



Scheme 2

CONCLUSIONS

Acid-catalyzed 'ring-switching' reactions of (*S*)-glutamic acid derived enaminone **1** with various monosubstituted hydrazines **2a-i** furnished a small library of 'ring-switched' products, *tert*-butyl (*S*)-*N*-Boc-3-(5-hydroxy-1*H*-pyrazol-4-yl)alaninates **3a-i**. Acid-catalyzed deprotection with a mixture of trifluoroacetic acid and dichloromethane furnished free amino acids **4** isolated as dihydrochloride salts. On the other hand, treatment of **3c**,**f** with HCl-EtOAc afforded the corresponding *N*-Boc deprotected *tert*-butyl esters dihydrochlorides **5a**,**b**. Basic hydrolysis of **3a** furnished *N*-Boc-protected amino acid **6**. In summary, the above method enables a simple preparation of free 3-pyrazolylalanines **4** and their partially protected derivatives **5** and **6** that are suitable for the incorporation into oligopeptides. Since a variety of heterocylic systems can be obtained by the reaction of enamino esters and amides with ambident nuclophiles,[1] the above method is also amenable for the preparation of other types of 3-heteroarylalanines and their partially protected derivatives.

EXPERIMENTAL

Solvents for extractions and chromatography were of technical grade and were distilled prior to use. Extracts were dried over technical grade Na_2SO_4. Melting points were determined on a Kofler micro hot stage and on SRS OptiMelt MPA100 – Automated Melting Point System (Stanford Research Systems, Sunnyvale, California, United States). The NMR spectra were obtained on a Bruker Avance DPX 300 at 300 MHz for ^{1}H and 75.5 MHz for ^{13}C nucleus, and on a Bruker UltraShield 500 plus (Bruker, Billerica, Massachusetts, United States) at 500 MHz for ^{1}H and 126 MHz for ^{13}C nucleus, using DMSO-d_6 and $CDCl_3$

with TMS as the internal standard, as solvents. Mass spectra were recorded on an Agilent 6224 Accurate Mass TOF LC/MS (Agilent Technologies, Santa Clara, California, United States), IR spectra on a Perkin-Elmer Spectrum BX FTIR spectrophotometer (PerkinElmer, Waltham, Massachusetts, United States). Microanalyses were performed on a Perkin-Elmer CHN Analyzer 2400 II (PerkinElmer, Waltham, Massachusetts, United States). Column chromatography (CC) was performed on silica gel (Silica gel 60, particle sofe: 0.035-0.070 mm (Sigma-Aldrich, St. Louis, Missouri, United States).

Hydrazine hydrochloride (**2a**), phenylhydrazine hydrochloride (**2b**) (4-nitrophenyl)hydrazine (**2c**), (4-fluorophenyl)hydrazine hydrochloride (**2d**), (4-chlorophenyl)hydrazine hydrochloride (**2e**), (3-chlorophenyl)hydrazine hydrochloride (**2f**), *o*-tolylhydrazine hydrochloride (**2g**), 2-hydrazinylpyrimidine (**2h**), and *tert*-butylhydrazine hydrochloride (**2i**) are commercially available (Sigma-Aldrich, St. Louis, Missouri, United States). (*S*,*E*)-Di-*tert*-butyl 4-[(dimethylamino)methylidene]-5-oxopyrrolidine-1,2-dicarboxylate (**1**)[21] was prepared following the literature procedure.

The source of chirality: L-pyroglutamic acid, product number 83160 Aldrich, assay: ≥99.0% (T), $[\alpha]_D^{20}$ −10.5±1 (c 5% in H_2O), mp 155-162 °C, optical purity: enantiomeric ratio ≥99:1 (GC).

Synthesis of Boc-protected *α*-amino acids 3a-i – *General procedure* (*GP1*).

To a solution of (*S*,*E*)-di-*tert*-butyl 4-((dimethylamino)methylene)-5-oxopyrrolidine-1,2-dicarboxylate (**1**) (1 equivalent) in *n*PrOH hydrazine hydrochloride **2** (1 equivalent) or hydrazine **2** (1 equivalent) and HCl (2M in EtOAc, 1 equivalent) was/were added and the resulting mixture was heated under reflux for 3-8 h. Volatile components were evaporated *in vacuo* and the residue was purified by column chromatography (CC) (Silica gel 60). Fractions containing the product were combined and volatile components evaporated *in vacuo* to give **3**.

(*S*)-*tert*-Butyl 2-((*tert*-butoxycarbonyl)amino)-3-(5-hydroxy-1*H*-pyrazol-4-yl)propanoate (3a)

Following *GP1*: Prepared from **1** (1.362 g, 4 mmol), ^{n}PrOH (10 mL), hydrazine hydrochloride (**2a**) (274 mg, 4 mmol), t = 4 h, CC (EtOAc). Yield: 1.210 g (92%) of yellowish solid; mp 74.9-82.0 °C. $[\alpha]_D^{rt}$ –7.33 (c 0.18, CH_2Cl_2). Anal. Calcd for $C_{15}H_{25}N_3O_5$: C, 55.03; H, 7.70; N, 12.84. Found C, 55.04; H, 7.68; N, 12.44. EI-HRMS: *m/z* = 328.1867 (MH^+); $C_{15}H_{26}N_3O_5$ requires: *m/z* = 328.1868 (MH^+); ν_{max} 3407, 2977, 1718, 1700, 1617, 1533, 1393, 1368, 1254, 1156, 1051, 850 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.41 (9H, *s*, *t*Bu); 1.42 (9H, *s*, *t*Bu); 2.78 – 2.88 (2H, *m*, CH_2); 4.13 (0.3H, br *s*, CH); 4.33 (0.7H, br *s*, CH); 5.65 (0.7H, br *s*, NH); 6.57 (0.3H, br *s*, NH); 7.17 (1H, *s*, CH); 8.21 (1H, br *s*, NH). ^{13}C-NMR (126 MHz, DMSO-d_6): δ 24.3, 27.6, 28.2, 54.9, 78.1, 80.1, 98.8, 129.0, 155.4, 159.3, 171.4.

(*S*)-*tert*-Butyl 2-((*tert*-butoxycarbonyl)amino)-3-(5-hydroxy-1-phenyl-1*H*-pyrazol-4-yl)propanoate (3b)

Following *GP1*: Prepared from **1** (681 mg, 2 mmol), ^{n}PrOH (10 mL), phenylhydrazine hydrochloride (**2b**) (289 mg, 2 mmol), t = 3 h, CC (EtOAc/petroleum ether/CH_2Cl_2 = 1:1:1). Yield: 363 mg (45%) of yellow

solid; mp 165-166 °C. $[\alpha]_D^{rt}$ ±0.0 (c 0.14, CH_2Cl_2). Anal. Calcd for $C_{21}H_{29}N_3O_5$: C, 62.51; H, 7.24; N, 10.41. Found C, 62.14; H, 7.37; N, 10.23. EI-HRMS: *m/z* = 404.2181 (MH^+); $C_{21}H_{30}N_3O_5$ requires: *m/z* = 404.2180 (MH^+); ν_{max} 3391, 2978, 1717, 1707, 1618, 1582, 1515, 1458, 1369, 1306, 1250, 1184, 1163, 759 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.46 (9H, *s*, *t*Bu); 1.49 (9H, *s*, *t*Bu); 2.81 (1H, *dd*, *J* = 2.4; 15.2 Hz, Ha of CH_2); 2.88 (1H, *dd*, *J* = 7.0; 15.3 Hz, Hb of CH_2); 4.17 (1H, br *s*, CH); 5.58 (1H, br *s*, NH); 7.23 – 7.32 (2H, *m*, CH, 1H of Ph); 7.40 – 7.45 (2H, *m*, 2H of Ph); 7.79 (2H, *d*, *J* = 7.1 Hz, 2H of Ph); 9.64 (1H, br *s*, NH). ^{13}C-NMR (126 MHz, DMSO-d_6): δ 24.6, 27.6, 28.2, 54.8, 78.1, 80.2, 120.4, 125.3, 128.9, 138.4, 139.7, 155.4, 171.3.

(*S*)-*tert*-Butyl 2-((*tert*-butoxycarbonyl)amino)-3-(5-hydroxy-1-(4-nitrophenyl)-1*H*-pyrazol-4-yl)-propanoate (3c)

Following *GP1*: Prepared from **1** (681 mg, 2 mmol), ^{n}PrOH (10 mL), (4-nitrophenyl)hydrazine (**2c**) (306 mg, 2 mmol), HCl (2M in EtOAc, 1 mL), t = 4 h, CC (EtOAc). Yield: 513 mg (57%) of yellow solid; mp 68.2-79.6 °C. $[\alpha]_D^{rt}$ –6.0 (c 0.10, CH_2Cl_2). Anal. Calcd for $C_{21}H_{28}N_4O_7$: C, 56.24; H, 6.29; N, 12.49. Found C, 56.11; H, 6.15; N, 12.22. EI-HRMS: *m/z* = 449.2031 (MH^+); $C_{21}H_{29}N_4O_7$ requires: *m/z* = 449.2032 (MH^+); ν_{max} 3396, 2982, 2930, 1735, 1686, 1636, 1613, 1595, 1512, 1447, 1369, 1330, 1305, 1251, 1156, 1112, 1052, 960, 852, 828, 749 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.48 (9H, *s*, *t*Bu); 1.50 (9H, *s*, *t*Bu); 2.80 (1H, *dd*, *J* = 2.2; 15.5 Hz, Ha of CH_2); 2.90 (1H, *dd*, *J* = 7.5; 15.5 Hz, Hb of CH_2); 4.11 (1H, br *s*, CH); 5.70 (1H, *d*, *J* = 4.1 Hz, NH); 7.36 (1H, *s*, CH); 8.10 – 8.17 (2H, *m*, 2H of Arl); 8.27 – 8.31 (2H, *m*, 2H of Arl); 10.40 (1H, br *s*, NH). ^{13}C-NMR (126 MHz, DMSO-d_6): δ 24.4, 27.6, 28.2, 54.4, 78.2, 80.3, 100.9, 119.2, 125.0, 142.1, 143.5, 143.6, 155.4, 171.2.

(*S*)-*tert*-Butyl 2-((*tert*-butoxycarbonyl)amino)-3-(1-(4-fluorophenyl)-5-hydroxy-1*H*-pyrazol-4-yl)-propanoate (3d)

Following *GP1*: Prepared from **1** (681 mg, 2 mmol), ^{n}PrOH (5 mL), (4-fluorophenyl)hydrazine hydrochloride (**2d**) (325 mg, 2.00 mmol), t = 3 h, CC (EtOAc/petroleum ether = 1:1). Yield: 767 mg (91%) of orange-brown solid; mp 147.8-148.6 °C. $[\alpha]_D^{rt}$ +8.2 (c 0.20, CH_2Cl_2). Anal. Calcd. for $C_{21}H_{28}FN_3O_5$: C, 59.85; H, 6.70; N, 9.97. Found C, 59.61; H, 6.50; N, 9.76. EI-HRMS: *m/z* = 422.2084 (MH^+); $C_{21}H_{29}FN_3O_5$ requires: *m/z* = 422.2086 (MH^+); ν_{max} 3391, 2980, 1704, 1621, 1588, 1510, 1368, 1313, 1300, 1254, 1230, 1160, 836, 754 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.46 (9H, *s*, *t*Bu); 1.48 (9H, *s*, *t*Bu); 2.79 (1H, *dd*, *J* = 2.5; 15.3 Hz, Ha of CH_2); 2.87 (1H, *dd*, *J* = 6.9; 15.2 Hz, Hb of CH_2); 4.13 – 4.19 (1H, *m*, CH); 5.63 (1H, br *s*, NH); 7.06 – 7.14 (2H, *m*, 2H of Arl); 7.27 (1H, *s*, CH); 7.68 – 7.70 (2H, *m*, 2H of Arl); 9.80 (1H, br *s*, NH). ^{13}C-NMR (126 MHz, DMSO-d_6): δ 24.5, 27.6, 28.2, 54.8, 78.1, 80.2, 98.0, 115.6, 115.7, 122.8, 135.2, 140.1, 149.5, 155.4, 158.6, 160.5, 171.3.

(*S*)-*tert*-Butyl 2-((*tert*-butoxycarbonyl)amino)-3-(1-(4-chlorophenyl)-5-hydroxy-1*H*-pyrazol-4-yl)-propanoate (3e)

Following *GP1*: Prepared from **1** (681 mg, 2 mmol), ^{n}PrOH (10 mL), (4-chlorophenyl)hydrazine hydrochloride (**2e**) (358 mg, 2.5 mmol), t = 4 h, CC (EtOAc/petroleum ether = 1:1). Yield: 552 mg (63%) of orange solid; mp 122-130 °C. $[\alpha]_D^{rt}$ +2.7 (c 0.30, CH_2Cl_2). Anal. Calcd for $C_{21}H_{28}ClN_3O_5$: C, 57.60; H, 6.44; N, 9.60. Found C, 57.42; H, 6.72; N, 9.53. EI-HRMS: *m/z* = 438.1794 (MH^+); $C_{21}H_{29}ClN_3O_5$ requires: *m/z* = 438.1790 (MH^+); ν_{max} 3382, 3113, 2978, 2936, 1705, 1622, 1580, 1494, 1369, 1343, 1302, 1250, 1157, 1084, 1054, 1014, 961, 846, 824, 791 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.46 (9H, s, *t*Bu); 1.48 (9H, s, *t*Bu); 2.79 (1H, *dd*, *J* = 3.1; 15.3 Hz, Ha of CH_2); 2.87 (1H, *dd*, *J* = 7.1; 15.3 Hz, Hb of CH_2); 4.14 (1H, *s*, CH); 5.62 (1H, br *s*, NH); 7.29 (1H, *s*, CH); 7.34 – 7.40 (2H, *m*, 2H of Arl); 7.76 (2H, *d*, *J* = 8.4 Hz, 2H of Arl); 9.87 (1H, br *s*, NH). ^{13}C-NMR (126 MHz, DMSO-d_6): δ 24.4, 27.6, 28.2, 54.7, 78.1, 80.2, 98.8, 122.1, 128.9, 129.4, 137.5, 140.4, 155.4, 171.3.

(*S*)-*tert*-Butyl 2-((*tert*-butoxycarbonyl)amino)-3-(1-(3-chlorophenyl)-5-hydroxy-1*H*-pyrazol-4-yl)-propanoate (3f)

Following *GP1*: Prepared from **1** (851 mg, 2.5 mmol), ^{n}PrOH (10 mL), (3-chlorophenyl)hydrazine hydrochloride (**2f**) (448 mg, 2.5 mmol), t = 4 h, CC (EtOAc/petroleum ether = 1:1). Yield: 663 mg (60%) of yellowish solid; mp 77.6-84.2 °C. $[\alpha]_D^{rt}$ +4.4 (c 0.31, CH_2Cl_2). Anal. Calcd for $C_{21}H_{28}ClN_3O_5$: C, 57.60; H, 6.44; N, 9.60. Found C, 57.36; H, 6.71; N, 9.55. EI-HRMS: *m/z* = 438.1802 (MH^+); $C_{21}H_{29}ClN_3O_5$ requires: *m/z* = 438.1790 (MH^+); ν_{max} 3550, 3462, 3411, 1719, 1633, 1618, 1594, 1483, 1438, 1393, 1368, 1254, 1153, 1104, 1052, 845, 780, 680, 620 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.46 (9H, *s*, *t*Bu); 1.48 (9H, *s*, *t*Bu); 2.78 (1H, *dd*, *J* = 2.8; 15.3 Hz, Ha of CH_2); 2.87 (1H, *dd*, *J* = 7.0; 15.3 Hz, Hb of CH_2); 4.14 (1H, *s*, CH); 5.65 (1H, br *s*, NH); 7.20 (1H, *d*, *J* = 8.0 Hz, 1H of Arl); 7.29 (1H, *s*, CH); 7.33 (1H, *t*, *J* = 8.1 Hz, 1H of Arl); 7.72 (1H, *d*, *J* = 7.6 Hz, 1H of Arl); 7.85 (1H, br *s*, 1H of Arl); 9.97 (1H, br *s*, NH). ^{13}C-NMR (126 MHz, $CDCl_3$): δ 26.7, 28.1, 28.4, 55.2, 80.5, 82.5, 101.6, 119.1, 121.1, 126.1, 130.0, 134.6, 138.5, 139.1, 156.1, 171.4.

(*S*)-*tert*-Butyl 2-((*tert*-butoxycarbonyl)amino)-3-(5-hydroxy-1-(*o*-tolyl)-1*H*-pyrazol-4-yl)propanoate (3g)

Following *GP1*: Prepared from **1** (124 mg, 0.365 mmol), ^{n}PrOH (1 mL), *o*-tolylhydrazine hydrochloride (**2g**) (58 mg, 0.365 mmol), t = 8 h, CC (EtOAc/petroleum ether = 1:1). Yield: 88 mg (57%) of yellow solid; mp 73.4-85.8 °C. $[\alpha]_D^{rt}$ +7.8 (c 0.10, CH_2Cl_2). EI-HRMS: *m/z* = 418.2338 (MH^+); $C_{22}H_{32}N_3O_5$ requires: *m/z* = 418.2336 (MH^+); ν_{max} 3417, 2980, 1716, 1636, 1616, 1497, 1390, 1368, 1251, 1154, 1050, 849, 760, 626 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.40 (9H, *s*, *t*Bu); 1.42 (9H, *s*, *t*Bu); 2.04 (3H, *s*, Me); 2.66 (1H, *dd*, *J* = 5.5; 15.0 Hz, Ha of CH_2); 2.71 (1H, *dd*, *J* = 5.1; 15.0 Hz, Hb of CH_2); 4.14 (1H, *dd*, *J* = 5.4, 11.4 Hz, CH); 5.99 (1H, br *s*, NH); 6.97 (1H, *s*, 1H of Arl); 7.10 (1H, *d*, *J* = 7.6 Hz, 1H of Arl); 7.17 (1H, deg *t*, *J* = 7.3; 7.6 Hz, 1H of Arl); 7.22 (1H, *d*, *J* = 7.3 Hz, 1H of Arl); 7.27 (1H, deg *t*, *J* = 7.2, 7.5

Hz, 1H of Arl); 10.41 (1H, br *s*, NH). ^{13}C-NMR (126 MHz, DMSO-d_6): δ 17.8, 26.7, 28.1, 28.5, 55.6, 77.4, 80.2, 82.2, 99.3, 126.5, 127.8, 129.2, 131.0, 135.8, 136.3, 137.6, 156.2, 171.7.

(*S*)-*tert*-Butyl 2-((*tert*-butoxycarbonyl)amino)-3-(5-hydroxy-1-(pyrimidin-2-yl)-1*H*-pyrazol-4-yl)-propanoate (3h)

Following *GP1*: Prepared from **1** (851 mg, 2.5 mmol), ^{n}PrOH (10 mL), 2-hydrazinylpyrimidine (**2h**) (275 mg, 2.5 mmol), HCl (2M in EtOAc, 1.25 ml), t = 8 h, CC (EtOAc/MeOH = 10:1). Yield: 285 mg (28%) of yellow solid; mp 68.5-79.7 °C. $[\alpha]_D^{rt}$ +13.1 (c 0.20, CH_2Cl_2). EI-HRMS: *m/z* = 406.2086 (MH^+); $C_{19}H_{28}N_5O_5$ requires: *m/z* = 406.2085 (MH^+); ν_{max} 3406, 2979, 1715, 1652, 1582, 1456, 1411, 1367, 1251, 1151, 1050, 1022, 971, 848, 797, 764, 701, 645 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.44 (9H, *s*, *t*Bu); 1.45 (9H, *s*, *t*Bu); 2.86 (1H, *dd*, *J* = 5.7; 14.9 Hz, Ha of CH_2); 2.94 (1H *dd*, *J* = 5.3; 14.8 Hz, Hb of CH_2); 4.40 (1H, *q*, *J* = 5.7 Hz, CH); 5.26 (1H, *d*, *J* = 8.0 Hz, NH); 7.24 (1H, *t*, *J*= 4.9 Hz, 1H of Arl); 7.47 (1H, *s*, CH); 8.74 (2H, *d*, *J* = 4.9 Hz, 1H of Arl); 11.60 (1H, br *s*, NH). ^{13}C-NMR (126 MHz, $CDCl_3$): δ 25.2, 28.0, 28.4, 54.2, 79.6, 82.0, 96.8, 117.8, 144.7, 153.7, 155.3, 157.2, 158.3, 170.9.

(*S*)-*tert*-Butyl 2-((*tert*-butoxycarbonyl)amino)-3-(1-(*tert*-butyl)-5-hydroxy-1*H*-pyrazol-4-yl)-propanoate (3i)

Following *GP1*: Prepared from **1** (681 mg, 2 mmol), ^{n}PrOH (10 mL), *tert*-butylhydrazine hydrochloride (**2i**) (249 mg, 2 mmol), t = 6 h, CC (EtOAc/petroleum ether = 1:1). Yield: 288 mg (37%) of yellowish solid; mp 53.3-59.3 °C. $[\alpha]_D^{rt}$ ±0.0 (c 0.09, CH_2Cl_2). Anal. Calcd for $C_{19}H_{33}N_3O_5$: C, 59.51; H, 8.67; N, 10.96. Found C, 59.73; H, 8.85; N, 10.84. EI-HRMS: *m/z* = 384.2495 (MH^+); $C_{19}H_{34}N_3O_5$ requires: *m/z* = 384.2493 (MH^+); ν_{max} 3406, 2976, 1718, 1633, 1617, 1522, 1458, 1394, 1368, 1250, 1157, 1054, 848, 764 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.46 (18H, *s*, 2x*t*Bu); 1.60 (9H, *s*, *t*Bu); 2.75 – 2.84 (2H, *m*, CH_2); 4.18 – 4.24 (1H, *m*, CH); 5.38 (1H, br *s*, NH); 7.07 (1H, *s*, CH); 8.57 (1H, br *s*, NH).

Synthesis of amino acid dihydrochlorides (4a-c) – *General procedure* (*GP2*).

To a solution of *N*-Boc-protected *α*-amino acid *tert*-butyl ester **3** (1 equivalent) in CH_2Cl_2 (10 ml) CF_3CO_2H (5 mL) was added and the resulting mixture was stirred at room temperature for 12 h. Volatile components were evaporated *in vacuo* and to the residue HCl (2 M in EtOAc, 15 mL) was added. The resulting mixture was stirred at room temperature till filterable precipitate was formed. The precipitate (product **4**) was collected by filtration, washed with anhydrous Et_2O (30 ml), dried on high vacuum, and stored under argon.

(*S*)-2-Amino-3-(5-hydroxy-1*H*-pyrazol-4-yl)propanoic acid dihydrochloride (4a)

Following *GP2*: Prepared from **3a** (379 mg, 1.13 mmol). Yield: 257 mg (93%) of white solid; mp 202-205 °C. $[\alpha]_D^{rt}$ ±0 (c 0.21, MeOH). EI-HRMS: *m/z* = 172.0714 (M^+); $C_6H_{10}N_3O_3^+$ requires: *m/z* = 172.0717 (M^+); ν_{max} 2885, 1731, 1620, 1605, 1580, 1478, 1429, 1376, 1338, 1293, 1268, 1225, 1138, 1081, 1048, 969, 912, 890, 857, 809, 751, 735, 676, 650 cm^{-1}. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 2.97 (1H,

dd, *J* = 7.2; 15.2 Hz, Ha of CH_2); 3.04 (1H, *dd*, *J* = 6.0; 15.2 Hz, Ha of CH_2); 4.08 (1H, *s*, CH); 7.84 (1H, *s*, CH); 8.56 (3H, *s*, NH_3); 12.35 (4H, br *s*). ^{13}C-NMR (126 MHz, DMSO-d_6): δ 23.0, 51.9, 98.0, 133.8, 156.0, 170.2.

(*S*)-2-Amino-3-(5-hydroxy-1-phenyl-1*H*-pyrazol-4-yl)propanoic acid dihydrochloride (4b)

Following *GP2*: Prepared from **3b** (279 mg, 0.691 mmol). Yield: 137 mg (62%) of brownish solid; mp 207-210 °C. $[\alpha]_D^{rt}$ –28.3 (c 0.48, MeOH). EI-HRMS: *m/z* = 248.1024 (M^+); $C_{12}H_{14}N_3O_3^+$ requires: *m/z* = 248.1030 (M^+); ν_{max} 3093, 3041, 2842, 2790, 2613, 1743, 1600, 1566, 1508, 1494, 1459, 1438, 1423, 1409, 1367, 1344, 1320, 1296, 1257, 1201, 1142, 1131, 1074, 1030, 942, 911, 846, 800, 757, 721, 701, 686, 650, 629, 617 cm^{-1}. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 2.99 (1H, *dd*, *J* = 6.9; 15.3 Hz, Ha of CH_2); 3.09 (1H, *dd*, *J* = 5.1; 15.3 Hz, Hb of CH_2); 4.16 (1H, *dd*, *J* = 5.5; 11.1 Hz, CH); 7.32 (1H, *t*, *J* = 7.4 Hz, 1H of Ph); 7.50 (2H, *dd*, *J* = 5.1; 10.8 Hz, 2H of Ph); 7.65 (1H, *s*, CH); 7.74 (2H, *dd*, *J* = 0.9; 8.5 Hz, 2H of Ph); 8.64 (3H, *d*, *J* = 4.0 Hz, NH_3); 9.74 (3H, br *s*). ^{13}C-NMR (126 MHz, DMSO-d_6): δ 24.0, 52.4, 98.1, 121.3, 126.3, 129.1, 137.3, 138.9, 155.2, 170.4.

(*S*)-2-Amino-3-(1-(4-fluorophenyl)-5-hydroxy-1*H*-pyrazol-4-yl)propanoic acid dihydrochloride (4c)

Following *GP1*: Prepared from **3d** (380 mg, 0.902 mmol). Yield: 230 mg (75%) of brownish solid; mp 177-180 °C. $[\alpha]_D^{rt}$ –29.8 (c 0.31, MeOH). EI-HRMS: *m/z* = 266.0936 (M^+); $C_{12}H_{13}FN_3O_3^+$ requires: *m/z* = 266.0935 (M^+); ν_{max} 2759, 2547, 1727, 1695, 1563, 1502, 1444, 1426, 1307, 1285, 1217, 1158, 1138, 1124, 1109, 1094, 1082, 1052, 1015, 933, 910, 894, 841, 819, 808, 782, 761, 741, 703, 686, 656, 633, 609 cm^{-1}. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 2.99 (1H, *dd*, *J* = 6.9; 15.2 Hz, Ha of CH_2); 3.10 (1H, *dd*, *J* = 5.0; 15.2 Hz, Hb of CH_2); 4.15 (1H, *d*, *J* = 5.3 Hz, CH); 7.34 (2H, *t*, *J* = 8.8 Hz, 2H of Arl); 7.60 (1H, *s*, CH); 7.76 (2H, *dd*, *J* = 4.9; 9.0 Hz, 2H of Arl); 8.61 (3H, *d*, *J* = 2.7 Hz, NH_3); 11.85 (3H, br *s*). ^{13}C-NMR (126 MHz, DMSO-d_6): δ 23.9, 52.4, 97.6, 115.8, 115.9, 123.4, 123.5, 134.00, 134.02, 139.1, 154.7, 159.1, 161.1, 170.5.

Synthesis of *N*-Boc deprotected *tert*-butyl ester dihydrochlorides (5a,b) – *General procedure* (*GP3*).

To a solution of *N*-Boc-protected *α*-amino acid *tert*-butyl ester **3** in EtOAc (3 mL) HCl in EtOAc (2 M, 1 mL) was added and the resulting mixture was stirred at room temperature till the disappearance for the starting ester **3** as judged by the TLC-analysis (EtOAc, *ca*. 4-6 h). To the suspension Et_2O (2 mL) was added, the reaction mixture was cooled to 0 °C, the precipitate was collected by filtration, and washed with Et_2O (5 mL). The resulting products **5** were dried on high vacuum and stored under Argon.

***tert*-Butyl (*S*)-2-amino-3-(5-hydroxy-1-(4-nitrophenyl)-1*H*-pyrazol-4-yl)propanoate dihydrochloride (5a)**

Following *GP3*: Prepared from **3c** (130 mg, 0.290 mmol). Yield: 40 mg (32%) of yellow solid; mp 180-186 °C. $[\alpha]_D^{rt}$ –35.5 (c 0.20, MeOH). EI-HRMS: *m/z* = 349.1501 (M^+); $C_{16}H_{21}N_4O_5^+$ requires: *m/z* = 349.1506 (M^+); ν_{max} 2979, 2800, 1740, 1600, 1568, 1527, 1495, 1426, 1395, 1370, 1345, 1297, 1261,

1154, 1128, 1069, 935, 852, 837, 769, 749, 685, 627 cm^{-1}. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 1.39 (*s*, 9H, *t*Bu); 2.93 (*d*, *J* = 6.6 Hz, 2H, CH_2), 4.14 (q, *J* = 5.9 Hz, 1H, CH); 7.73 (*s*, 1H, CH); 8.06 – 8.13 (*m*, 2H, 2H of Arl); 8.33 – 8.39 (*m*, 2H, 2H of Arl); 8.52 (br *s*, 3H, NH_3^+). ^{13}C-NMR (126 MHz, DMSO-d_6): δ 24.0, 27.5, 52.3, 82.8, 98.7, 119.4, 125.0, 141.9, 143.2, 143.8, 168.0.

***tert*-Butyl (*S*)-2-amino-3-(1-(3-chlorophenyl)-5-hydroxy-1*H*-pyrazol-4-yl)propanoate dihydrochloride (5b)**

Following *GP3*: Prepared from **3f** (157 mg, 0.359 mmol). Yield: 80 mg (54%) of dirty-white solid; mp 138-144 °C. $[\alpha]_D^{rt}$ –27.7 (c 0.36, MeOH). EI-HRMS: *m/z* = 338.1259 (M^+); $C_{16}H_{21}ClN_3O_3^+$ requires: *m/z* = 338.1266 (M^+); ν_{max} 2979, 2787, 2491, 1736, 1592, 1567, 1497, 1476, 1417, 1370, 1305, 1285, 1251, 1230, 1158, 1126, 1097, 1078, 966, 907, 838, 821, 784, 755, 734, 698, 680 cm^{-1}. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 1.39 (*s*, 9H, *t*Bu); 2.92 (*d*, *J* = 6.3 Hz, 2H, CH_2); 4.08 – 4.17 (*m*, 1H, CH); 7.33 (*ddd*, *J* = 0.9; 2.1; 8.1 Hz, 1H, 1H of Arl); 7.51 (*t*, *J* = 8.1 Hz, 1H, 1H of Arl); 7.64 (*s*, 1H, CH); 7.74 (*ddd*, *J* = 1.0; 2.1; 8.3 Hz, 1H, 1H of Arl); 7.85 (*t*, *J* = 2.0 Hz, 1H, 1H of Arl); 8.55 (br *d*, *J* = 4.8 Hz, 1H, NH_3^+). ^{13}C-NMR (126 MHz, DMSO-d_6): δ 24.1, 27.5, 52.5, 82.8, 98.2, 118.5, 119.6, 125.3, 130.8, 133.3, 139.2, 140.2, 155.7, 168.0.

Synthesis of (*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-(5-hydroxy-1*H*-pyrazol-4-yl)propanoic acid (6)

To a suspension of pyrazole **3a** (280 mg, 0.855 mmol) in a mixture of H_2O (2 mL) and MeOH (0.5 mL), $LiOH \cdot H_2O$ (238 mg, 5.67 mmol) was added and the resulting mixture was stirred at room temperature for 3 h. Then, HCl (1 M in H_2O, 5.7 mL) was added and the resulting mixture was extracted with EtOAc (3×50 mL). Combined organic phases were dried over anhydrous Na_2SO_4, filtered, and volatile components evaporated *in vacuo* to give *N*-Boc protected amino acid **6**. Yield: 162 mg (69%) of colorless solid; mp 70-73 °C. $[\alpha]_D^{rt}$ –13.3 (c 0.22, MeOH). EI-HRMS: *m/z* = 272.1237 (MH^+); $C_{11}H_{18}N_3O_5$ requires: *m/z* = 272.1241 (MH^+); ν_{max} 3108, 2977, 2931, 1678, 1589, 1509, 1392, 1366, 1244, 1157, 1047, 1023, 850, 775, 751, 635 cm^{-1}. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 1.35 (9H, *s*, *t*Bu); 2.52 (1H, *dd*, *J* = 9.6; 14.6 Hz, Ha of CH_2); 2.69 (1H, *dd*, *J* = 4.7; 14.6 Hz, Hb of CH_2); 3.97 (1H, *td*, *J* = 4.9; 9.2 Hz, CH); 6.91 (1H, *d*, *J* = 8.0 Hz, NH); 7.19 (1H, *s*, CH); 11.38 (3H, br *s*, OH, NH, COOH). ^{13}C-NMR (126 MHz, DMSO-d_6): δ 24.2, 28.2, 54.2, 78.0, 99.1, 128.9, 155.4, 159.2, 173.8.

ACKNOWLEDGEMENTS

The financial support from Boehringer-Ingelheim Pharma (Biberach, Germany) and from the Slovenian Research Agency through grant P1-0179 is gratefully acknowledged.

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