Chemo- and regioselective modification of D,L-phenylalanine with α-cyanoacetylenic alcohols in water

Anastasiya G. Mal'kina, Angela P. Borisova, Valentina V. Nosyreva, Olesya A. Shemyakina, Alexander I. Albanov, and Boris A. Trofimov*

A. E. Favorsky Irkutsk Institute of Chemistry, Siberian Branch, Russian Academy of Sciences, 1 Favorsky Str., 664033, Irkutsk, Russia
E-mail: boris_trofimov@irioch.irk.ru

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Abstract

D,L-Phenylalanine reacts with α-cyanoacetylenic alcohols under biomimetic conditions (room temperature, water) to give a novel family of unnatural amino acids, containing 2,5-dihydro-5-iminofuranyl substituents in the amino group, in almost quantitative yields (92-95%). This "green" one-pot version of the tandem assembly of nontraditional amino acids opens an unexplored avenue to the new types of drugs and their precursors.

Keywords: D,L-Phenylalanine; α-cyanoacetylenic alcohols; 2,5-dihydroiminofurans; nucleophilic addition

Introduction

Among the amino acids, a special place belongs to their aromatic congeners, in particular, phenylalanine.[1] The phenylalanine derivatives are used as antidiabetic agents (nateglinide).[2] Among them there are ACE-NEP,[3] urokinase[4] and pyruvate kinase inhibitors.[5] Modifications of phenylalanine are active against the infections caused by Gram-positive bacteria (e.g. vancomycin[6]). Some of them are applicable for the treatment of rheumatoid arthritis, inflammatory bowel diseases, systemic lupus erythematosus, multiple sclerosis, Sjögren's syndrome, asthma, psoriasis, allergy, cardiovascular diseases, arterial sclerosis, restenosis, tumor proliferation and transplantation rejection.[7]

Therefore, the development of new synthetic strategies for phenylalanine modification is of essential importance. Meanwhile, the chemo- and regioselective transformation of the phenylalanine structural unit still represents a certain challenge and remains a subject of steadily growing investigations.

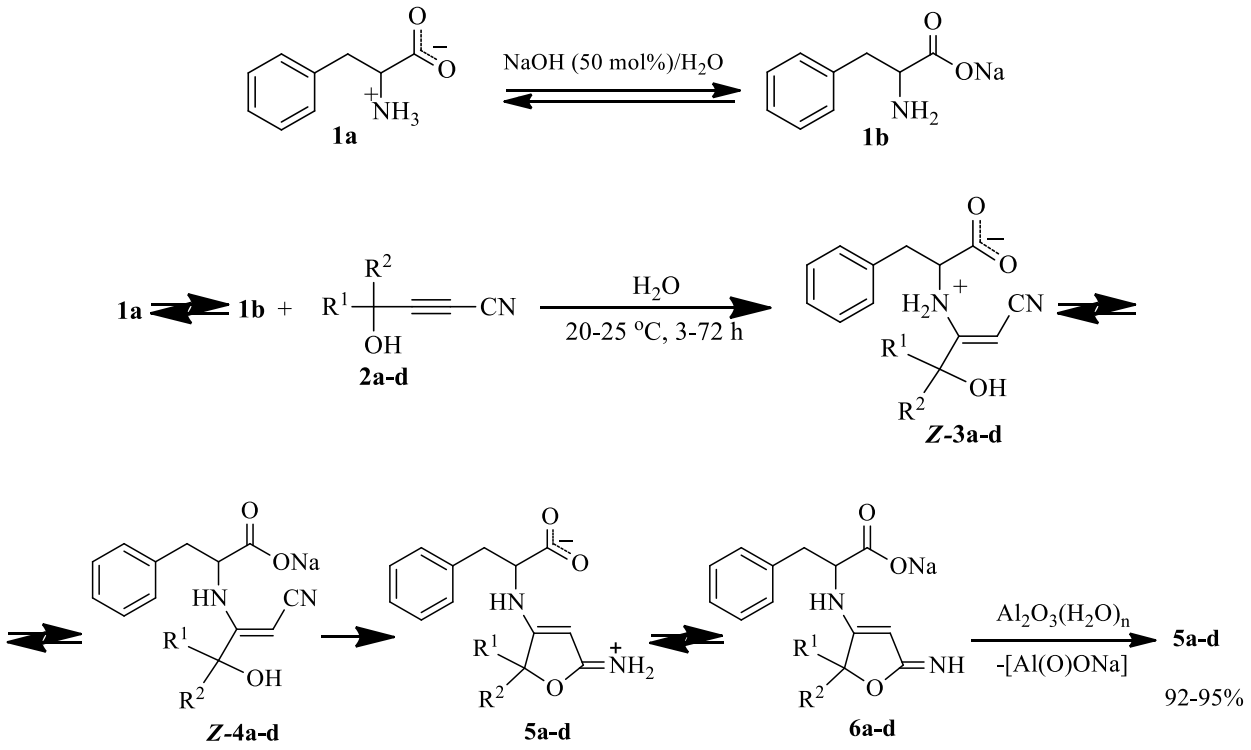
In this line, regioselective postsynthetic modification of phenylalanine residue with IPy_2BF_4 was reported.[8] An efficient arylation of phenylalanine side chains (in unprotected iodopeptides)

was rendered by Suzuki-Miyaura cross-coupling.[9] Recently, a microwave-assisted esterification of *N*-acetyl-L-phenylalanine with modified Mukaiyama's reagents was also published.[10] A series of fullerene substituted phenylalanine derivatives was prepared from 1,2-(4′-oxocyclohexano)fullerene and protected (4-amino)phenylalanine.[11] The RAFT-polymerization of *N*-acryloyl-L-phenylalanine was accomplished.[12]

This concise but not exhaustive overview of the latest results concerning the phenylalanine modification shows that the efforts in this area keep extending. The objective of this paper is two-fold. The first is to check whether the strategy for the modification of amino acids with α-cyanoacetylenic alcohols (α,β-acetylenic γ-hydroxy nitriles) recently pioneered by us[13] is general and also valid for phenylalanine. The second one is, using this strategy, to elaborate the efficient chemo- and regioselective synthesis of novel family of unnatural amino acids of unusual structure.

Results and Discussion

As applied to D,L-phenylalanine **1**, the above strategy implies the reacting of these amino acids with available[14] α-cyanoacetylenic alcohols **2a-d** to afford the initial adducts **3a-d** and **4a-d** which might be capable of further cyclizing to iminodihydrofuran substituted amino acids **5a-d** (Scheme 1).



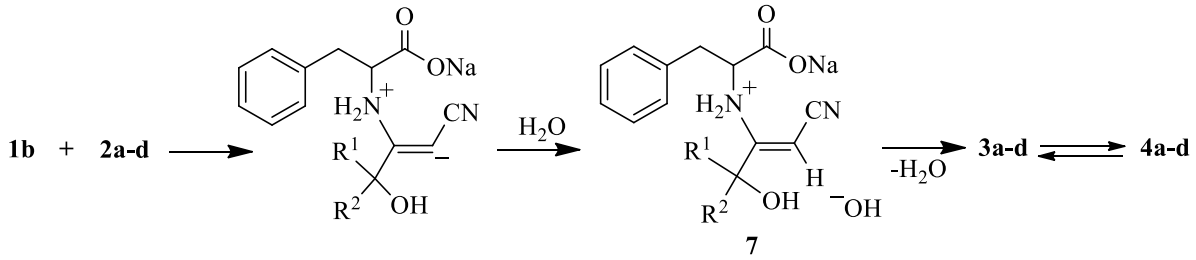
Scheme 1. Synthesis of unnatural amino acids **5a-d.**

We have started with the monitoring of the reaction conditions which has shown that the above Scheme 1 is actually realizable when the reactants **1** and **2** are allowed to contact in aqueous medium in the presence of 50 mol% of NaOH (pH ~8-9) at room temperature, i.e. under biomimetic conditions. The modified phenylalanines **5a-d** are formed chemo- and regioselectively in almost quantitative yields (92-95%), Scheme 1.

As depicted in Scheme 1, in the presence of 50 mol% of NaOH, zwitterionic form of phenylalanine **1a** is partially neutralized to deliver carboxylate **1b** having free amino group. The latter nucleophilically attacks α-cyanoacetylenic alcohols **2a-d** to give two primary adducts, correspondently zwitterions **3a-d** and carboxylates **4a-d**, both being in equilibrium. Then (after adopting *E*-configuration) follows their cyclization to iminodihydrofurans **5a-d** and **6a-d** which are also in equilibrium. Finally, the equilibrium mixtures of iminodihydrofurans **5a-d** and **6a-d** were passed through alumina, $Al_2O_3(H_2O)_n$, to afford pure amino acids **5a-d**. Alumina is known[15] to possess Lewis and Bronsted acid centers, owing to which the salts **6a-d** exchange their sodium cation for proton.

The iminodihydrofuranyl moieties are assembled involving the cyano and hydroxyl functions of the primary adducts *Z*-**3a-d** and *Z*-**4a-d**. The *Z*-configuration of the adducts is predicted by the known *trans*-mode of nucleophilic addition to mono-substituted acetylenes.[16] Therefore, before cyclization, the adducts *Z*-**3a-d** and *Z*-**4a-d** should undergo the isomerization to *E*-isomers since *Z*-configuration is incapable of the ring closing.

As it is clear from the above consideration, the role of NaOH as a catalyst is to deprotonate the ammonium site of the amino acid **1a** thus converting it into a true nucleophile, salt **1b**, Scheme 2.



Scheme 2

The attack of the amino group of salt **1b** at acetylenes **2a-d** is accompanied by the concerted proton transfer (either from water molecules or intramolecularly from the hydroxyl group) to give the ammonium hydroxides **7** which after release of water molecule leads to adducts **3a-d**.

Duration of the synthesis depends on the structure of starting acetylenes **2a-d**: for acetylenes **2a**,**b** the reaction takes 3 hours, while acetylenes with cycloalkyl substituents **2c**,**d** react completely for 72 hours (control TLC). This is likely resulted from decreased solubility of these acetylenes in water and the steric hindrance to the reactants contact imposed on the reaction by the cyclic substituents.

Amino acids **5a-d** are light-yellow powders, soluble in water, methyl and ethyl alcohols; insoluble in most organic solvents.

Multinuclear ^{1}H, ^{13}C and 2D (HMBC) NMR spectroscopy data as well as IR and UV investigation results of the adducts **5a-d** are in agreement with their structures. In the ^{1}H NMR spectra of the adducts **5a-d**, there is an olefinic proton (H-3) signal at 4.74-4.81 ppm. The protons of the CH- and CH_2-groups form three double doublets of three-spin system ABX in the region 3.92-3.99, 3.11-3.33 and 2.86-2.96 ppm, respectively, protons of the phenyl ring are shown as a multiplet at 7.08-7.27 ppm. In the case of **5b**, the doubling of alkyl and olefinic hydrogen signals, corresponding to diastereomeric mixture *R*,*R*(*S*,*S*):*R*,*S*(*S*,*R*) = 1:1, is observed. The ^{13}C NMR spectra confirm also the structure of amino acids **5a-d**.

Three near-located carbon signals of the COO^-, HN-C=CH and $C=^+NH_2$ groups have been assigned using HMBC (^{1}H-^{13}C) technique. The 2D spectra of **5a-d** show the cross-peaks between signals of: olefinic proton (H-3) and iminodihydrofuranyl fragment (C-2); methyl group protons and carbon atom (C-4); olefinic proton and quaternary carbon atom (C-5) of the iminodihydrofuranyl cycle (Figure 1).

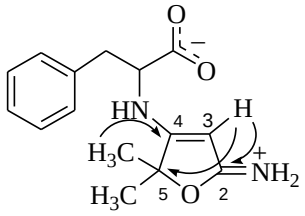


Figure 1. Cross-peaks in the 2D HMBC (^{1}H-^{13}C) spectrum of the amino acid **5a**.

Zwitterionic form of amino acids **5a-d** is supported by its IR (KBr) spectra where the broad strong absorption in the region of 3500-2600 cm^{-1} (with maxima 3424-3399, 3260-3219, 3085-3028, 2981-2862 cm^{-1}) is observed. These absorptions were commonly assigned[17] to NH, $=^+NH_2$, C=CH, CH groups. Carboxylate anion is spectrally manifested by a broad absorption in the region of 1700-1500 cm^{-1} probably overlapping with C=C bond stretching vibration at 1619-1615 cm^{-1} and deformation vibration $=^+NH_2$ at 1572-1559 cm^{-1}.

The unusual zwitterionic structure of amino acids **5a-d** follows from our recent results on the modification of glycine,[13a] 2-aminobenzoic acid,[13c] D,L-tryptophan[13d] using the same strategy, generality and feasibility of which are additionally confirmed by this work.

Conclusions

In summary, "green" chemo- and regioselective modification of D,L-phenylalanine with α-cyanoacetylenic alcohols in aqueous medium has been effected to furnish a new family of unnatural amino acids, containing 2,5-dihydro-5-iminofuranyl substituents, in almost

quantitative yields. The results obtained confirm the generality and feasibility of the environmentally benign strategy for unusual modification of the amino acids which allows to combine in a one molecule both amino acid and iminodihydrofuran pharmacophoric moieties. Such a strategy as well as the modified phenylalanine derivatives may find applications as prospective pharmaceuticals and precursors for drug design.

Experimental Section

General. ^{1}H and ^{13}C NMR spectra of the studied compounds were recorded in CD_3OD at 20-25 ^{o}C on Bruker DPX-400 spectrometer (400.13 and 100.62 MHz, respectively). NMR signals were assigned using 2D (HMBC ^{1}H-^{13}C) NMR technique. IR spectra were measured on a Bruker Vertex-70 instrument in KBr pellets. All melting points were taken on a Kofler micro hot stage. UV/Vis spectra were measured on a Perkin-Elmer Lambda 35 spectrometer at room temperature (EtOH, c 1.8-2.0·10^{-4} mol/L, d 0.1, 1.0 cm). Elemental analyses were perfomed on a FLASH EA 1112 Series instrument. The reaction was controlled by TLC on neutral Al_2O_3 (chloroform-benzene-ethanol, 20:4:1 as eluent). D,L-Phenyalanine **1** is commercial reagent ("Merck"). α-Cyanoacetylenic alcohols **2a-d** were prepared according to a published method.[14a,d]

General procedure for nucleophilic addition of D,L-phenylalanine (1) to α-cyanoacetylenic alcohols (2a-d)

To a solution of D,L-phenylalanine **1** (0.165 g, 1.0 mmol) in water (5 mL) and sodium hydroxide (50 mol%) in water (2 mL), appropriate acetylenes **2a-d** (1.0 mmol) were added. The mixture was stirred at room temperature for 3 hours (for **2a**,**b**) and 72 hours (for **2c**,**d**), stirring was stopped, and the water was evaporated. The residue obtained was passed through neutral Al_2O_3 [2-3 cm, eluent: 50-70 mL of hot ethanol (50-60 ºC)]. The solvent was evaporated under reduced pressure to give the amino acids **5a-d**.

2-[(5-Iminio-2,2-dimethyl-2,5-dihydro-3-furanyl)amino]-3-phenylpropanoate (5a). Light-yellow powder, yield 95%, 0.260 g, mp 190-192 ºC (dec.); IR (ν_{max}, cm^{-1}): 1559-1680 with maxima 1572 (δ $=^{+}NH_2$), 1619 (C=C), 1680 (C=O), 2600-3500 with maxima 2873, 2933, 2981 (CH), 3029, 3060, 3085 (C=CH), 3223, 3407 (NH, $=^{+}NH_2$). UV/Vis (EtOH): λ_{max} (log ε) = 274 nm (4.37). ^{1}H NMR (400.13 MHz, CD_3OD): δ_H 1.40 and 1.56 (6H, s, CH_3), 2.96, 3.33, 3.96 (3H, ABX, dd, $^{2}J_{AB}$ = 13.9, $^{3}J_{AX}$ = 4.4 Hz, $^{3}J_{BX}$ = 9.8 Hz, CH_2C^*H), 4.74 (1H, s, H-3), 7.16-7.19 (5H, m, Ph). ^{13}C NMR (100.62 MHz, CD_3OD): δ_C 24.6 and 25.2 (CH_3), 39.5 ($\underline{C}H_2C^*H$), 64.4 ($CH_2\underline{C}^*H$), 76.7 (C-3), 92.3 (C-5), 127.7 (C_p), 129.4, 130.4 ($C_{o,m}$), 139.2 (C_i), 176.0 ($C=^{+}NH_2$), 177.5 (HN-$\underline{C}$=CH), 178.3 (COO^{-}). Anal. Calcd for $C_{15}H_{18}N_2O_3$ (274.13): C, 65.69; H, 6.57; N, 10.20. Found: C, 65.40; H, 6.73; N, 10.38.

2-[(2-Ethyl-5-iminio-2-methyl-2,5-dihydro-3-furanyl)amino]-3-phenylpropanoate (5b). Light-yellow powder, yield 93%, 0.268 g, mp 182-184 ºC (dec.); IR (ν_{max}, cm^{-1}): 1559-1680 with maxima 1559 (δ $=^{+}NH_2$), 1618 (C=C), 1679 (C=O), 2600-3500 with maxima 2922, 2976 (CH),

3029, 3068 (C=CH), 3260, 3424 (NH, $=^{+}NH_2$). UV/Vis (EtOH): λ_{max} (log ε) = 274 nm (4.41). ^{1}H NMR (400.13 MHz, CD_3OD): δ_H 0.27 and 0.59 (6H, t, ^3J = 7.4 Hz, $CH_2C\underline{H_3}$), 1.24 and 1.38 (6H, s, CH_3), 1.68, 1.72 (2H, dq, ^2J = 15.1 Hz, $C\underline{H_2}CH_3$), 1.48, 1.79 (2H, dq, ^2J = 14.6 Hz, $C\underline{H_2}CH_3$), 2.83, 3.15, 3.91 (3H, ABX, dd, $^2J_{AB}$ = 10.0, $^3J_{AX}$ = 5.6, $^3J_{BX}$ = 7.7 Hz, CH_2C^*H), 2.86, 3.11, 3.92 (3H, ABX, dd, $^2J_{AB}$ = 9.0, $^3J_{AX}$ = 5.1, $^3J_{BX}$ = 7.7 Hz, CH_2C^*H), 4.76 and 4.81 (2H, s, H-3), 7.08-7.19 (5H, m, Ph). ^{13}C NMR (100.62 MHz, CD_3OD): δ_C 5.9 and 6.1 ($CH_2\underline{C}H_3$), 22.4 and 22.8 (CH_3), 30.0 and 30.2 ($\underline{C}H_2CH_3$), 37.3 and 37.4 ($\underline{C}H_2C^*H$), 62.3 and 62.6 ($CH_2\underline{C}^*H$), 77.6 and 77.7 (C-3), 94.0 (C-5), 126.8 (C_p), 128.5, 128.6, 128.9, 129.2 ($C_{o,m}$), 137.1 and 137.3 (C_i), 176.5 and 176.7 (COO^-), 176.3 and 176.4 (HN-$\underline{C}$=CH), 175.4 and 175.6 ($C=^{+}NH_2$). Anal. Calcd for $C_{16}H_{20}N_2O_3$ (288.35): C, 66.65; H, 6.99; N, 9.72. Found: C, 66.86; H, 7.23; N, 10.02.

2-[(2-Iminio-1-oxaspiro[4.4]non-3-en-4-yl)amino]-3-phenylpropanoate (5c). Orange powder, yield 92%, 0.276 g, mp 192-194 ºC (dec.); IR (ν_{max}, cm^{-1}): 1559-1680 with maxima 1569 (δ $=^{+}NH_2$), 1616 (C=C), 1678 (C=O), 2600-3500 with maxima 2874, 2957 (CH), 3031, 3059 (C=CH), 3221, 3401 (NH, $=^{+}NH_2$). UV/Vis (EtOH): λ_{max} (log ε) = 275 nm (4.49). ^{1}H NMR (400.13 MHz, CD_3OD): δ_H 1.81-2.19 [8H, m, $(CH_2)_4$], 2.96, 3.33, 3.99 (3H, ABX, dd, $^2J_{AB}$ = 13.7, $^3J_{AX}$ = 4.2, $^3J_{BX}$ = 9.5 Hz, CH_2C^*H), 4.81 (1H, s, H-3), 7.13-7.27 (5H, m, Ph). ^{13}C NMR (100.62 MHz, CD_3OD): δC 25.5, 25.6, 39.2, 39.3 (CH_2), 38.3 ($\underline{C}H_2C^*H$), 64.2 ($CH_2\underline{C}^*H$), 78.1 (C-3), 102.1 (C-5), 127.7 (C_p), 129.4, 130.4 ($C_{o,m}$), 139.2 (C_i), 175.6 ($C=^{+}NH_2$), 176.1 (HN-$\underline{C}$=CH), 177.4 (COO^-). Anal. Calcd for $C_{17}H_{20}N_2O_3$ (300.35): C, 67.98; H, 6.71; N, 9.33. Found: C, 67.68; H, 6.73; N, 9.38.

2-[(2-Iminio-1-oxaspiro[4.5]dec-3-en-4-yl)amino]-3-phenylpropanoate (5d). Light-yellow powder, yield 94%, 0.295 g, mp 180-182 ºC (dec.); IR (ν_{max}, cm^{-1}): 1559-1680 with maxima 1568 (δ $=^{+}NH_2$), 1615 (C=C), 1682 (C=O), 2600-3500 with maxima 2862, 2936 (CH), 3028, 3062 (C=CH), 3219, 3399 (NH, $=^{+}NH_2$). UV/Vis (EtOH): λ_{max} (log ε) = 274 nm (4.44). ^{1}H NMR (400.13 MHz, CD_3OD): δ_H 1.33-1.96 [10H, m, $(CH_2)_5$], 2.95, 3.32, 3.95 (3H, ABX, dd, $^2J_{AB}$ = 13.9, $^3J_{AX}$ = 4.5, $^3J_{BX}$ = 9.9 Hz, CH_2C^*H), 4.74 (1H, s, H-3), 7.12-7.27 (5H, m, Ph). ^{13}C NMR (100.62 MHz, CD_3OD): δ_C 23.0, 23.1, 25.0, 34.2, 34.9 (CH_2), 39.5 ($\underline{C}H_2C^*H$), 64.3 ($CH_2\underline{C}^*H$), 77.1 (C-3), 94.0 (C-5), 127.8 (C_p), 129.5, 130.6 ($C_{o,m}$), 139.2 (C_i), 176.1 ($C=^{+}NH_2$), 177.8 (HN-$\underline{C}$=CH), 178.3 (COO^-). Anal. Calcd for $C_{18}H_{22}N_2O_3$ (314.38): C, 68.77; H, 7.05; N, 8.91. Found: C, 68.47; H, 6.83; N, 8.68.

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