https://doi.org/10.15407/dopovidi2025.05.013
UDC 547.85:547.466.23

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Inverse electron demand Diels–Alder reaction of tetrazines and functionalized alkynes: convenient way to heterocycles and noncanonical amino acids

Presented by Academician of the NAS of Ukraine V.E. Kuzmin

A simple preparative synthesis of the heterocyclic system tetrahydropyrido[3,4-c]pyridazine has been developed within the framework of the IEDDA reaction of sym-tetrazine with methyl 2-(tert-butoxycarbonylamino)pent-4-ynoate. A series of pyridazinyl-α-alanines with different numbers of carboxyl groups — new non-canonical amino acids for bioengineering — have been obtained by its stepwise hydrolysis.

Keywords: *IEDDA reaction, tetrazine, 2-aminopent-4-ynoate, tetrahydropyrido[3,4-c]pyridazine, pyridazinyl-α-alanines, noncanonical aminoacids.*

Introduction. The search for useful but as yet unexplored chemical space is part of the concept of modern medicinal chemistry. Modern medicine and pharmacology face various challenges, including the efficacy and specificity of biologically active substances. The requirements for low toxicity, predictability of direct and side effects are increasing, and as a consequence, there is a need for new molecules, mainly in non-benzene heterocyclic systems. An important means of achieving clinical success is considered to be an increase in sp^3-saturation of active molecules, and not only because of greater solubility in aqueous media. All biological targets have a three-dimensional structure, therefore the specificity and selectivity of the action of new drugs is associated with their non-planar geometry. In the work [1] three criteria for success were proposed:

- (bi)heterocyclic structure of the lead molecule;

Citation: Svaljavin O.V., Bohdan N.M., Nechaieva D.S., Bohza S.L. Inverse electron demand Diels–Alder reaction of tetrazines and functionalized alkynes: convenient way to heterocycles and noncanonical amino acids. *Dopov. Nac. akad. nauk Ukr.* 2025. No 5. P. 13—18. https://doi.org/10.15407/dopovidi2025.05.013

▪ ratio of the total number of sp^3 hybridized carbon atoms to the total number of carbon atoms in the molecule;

▪ presence of a chiral center in the drug candidate.

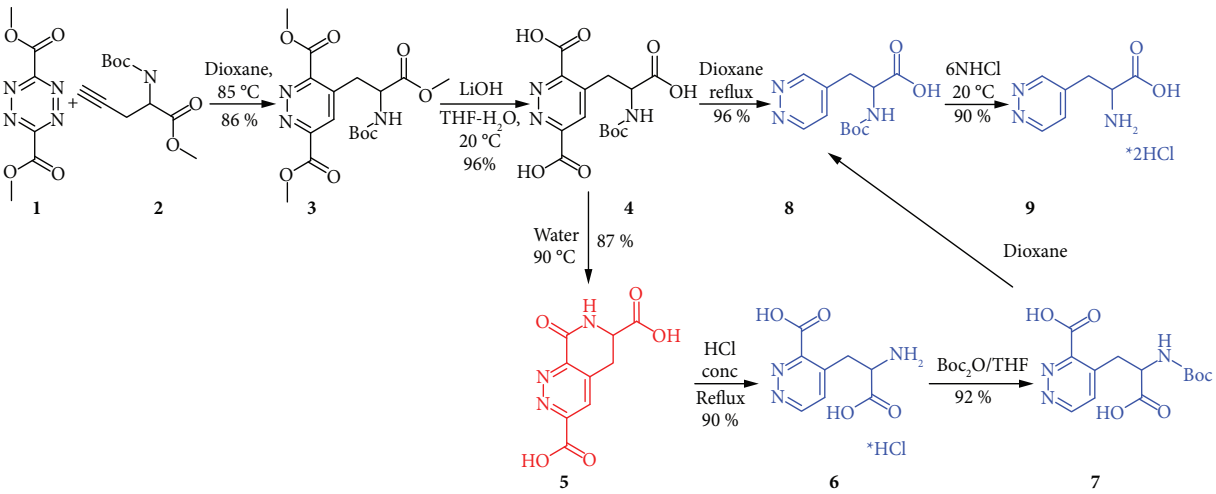
Such motifs provide improved physicochemical, toxicological, and medicinal properties on the molecule, often including improved target specificity, partly due to an increase in non-planar volume compared to (hetero)aromatic systems. In extension the search new bicyclic heterocycles with the maximum number of sp^3 atoms, our attention was drawn to the structure of pyrido[3,4-*c*] pyridazine. Among the variety of bicyclic nitrogen-containing heterocycles, pyrido[3,4-*c*]pyridazine is a rare subclass of compounds. This pyridopyridazine isomer was included in the virtual list of the so-called "heteroaromatic molecules of the future" in the review [2]. In 2022, a review of methods for pyrido[3,4-*c*]pyridazines synthesis was published [3], which emphazised their insufficient study and synthetic availability.

Approaches to the construction of pyrido[3,4-*c*]pyridazine framework, published in 23 articles over the 60 years since the first publication [4] [ref. 3], are based on the sequential build-up of substituents to the pyridine or pyridazine core and subsequent final cyclization with bicyclic system formation. These synthetic schemes are multistage, and the yields of the final products are usually low. One of the first syntheses of a pyrido[3,4-*c*]pyridazine derivative was the Diels–Alder reaction of 1,2,4,5-tetrazine-3,6-dicarboxylate and 2-R-pyrrolines as dienophiles [5]. The inverse electron demand Diels–Alder (IEDDA) reaction is attractive due to the possibility of forming condensed systems using functional derivatives of 1,2,4,5-tetrazine due to a significant reduction in the reaction pathway to the key stage. However, the reaction of tetrazines and cyclic imines is not of a general nature, since the structure of the final products critically depends on the substituents in the cyclic azomethine. At the same time, in our opinion, the IEDDA reaction can serve as a convenient basis for the pyrido[3,4-*c*]pyridazine system design. The reaction of 1,2,4,5-tetrazines and alkynes with the corresponding functional groups can be convenient. Unlike C=C- and C=N- dienophiles [2, 6], the presence of a triple bond as a dienophile ensures the possibility of formation an aromatic pyridazine ring and excludes the stage of aromatization of the dihydropyridazine cycle. Substituents in pyridazine and alkyne should ensure the formation of a pyridine ring with a minimum reaction steps.

The objective of our study is to find an efficient and simple approach to constructing a partially saturated pyrido[3,4-*c*]pyridazine skeleton with various functional groups using the IEDDA reaction.

Results and discussion. To obtain pyrido[3,4-*c*]pyridazine framework in the IEDDA methodology, we studied the reaction of dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate (**1**) with methyl 2-(tert-butoxycarbonylamino)pent-4-ynoate (**2**). It was found that heating equimolar amounts of compounds **1** and **2** in dioxane for 16 hours leads to the formation of dimethyl 4-(2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)pyridazine-3,6-dicarboxylate (**3**) in 86 % yield without use of catalysts. The presence of a protective Boc-group on the nitrogen atom and ester groups of pyridazine has did not allow further cyclization. Therefore, selective hydrolysis of the ester groups of pyridazine with lithium hydroxide in a THF – water medium at room temperature was developed. As a result, 4-(2-((tert-butoxycarbonyl)amino)-2-carboxyethyl)pyridazine-3,6-dicarboxylic acid (**4**) was obtained in 96 % yield.

Cyclization of tricarboxylate **4** proceeded surprisingly easily, leading to the expected tetrahydropyrido[3,4-*c*]pyridazine **5**, which was isolated in 87 % yield after heating in water at 90 °C. The structure of compound **5** was confirmed by NMR spectroscopy and LC-MS analysis



The synthesis of tetrahydropyrido[3,4-*c*]pyridazine **5** and its transformation into pyridazinyl-α-alanines

(see experimental section). In the strong-field part of the ^{1}H NMR spectrum in DMSO-d_6 there is a system of multiplets of the C-H, N-H groups and magnetically nonequivalent protons of the CH_2 group of the pyridine ring of pyrido[3,4-*c*]pyridazine. The signal of the amide N-H proton and carboxyl groups is located in the region of exchangeable protons of residual solvent water, in contrast to compounds 3 and 4, in which the Boc-N-H proton is present as a doublet in the range of 7.2—7.4 ppm. with a coupling constant of 9 Hz (in the experimental part).

When pyrido[3,4-*c*]pyridazine **5** was heated in hydrochloric acid, ring opening of the pyridine moiety occured, accompanied by partial decarboxylation of the pyridazine ring, leading to the formation of 4-(2-amino-2-carboxyethyl)pyridazine-3-carboxylic acid (6). The resulting 3-pyridazinyl-substituted α-alanine derivative **6** represents a new member of the noncanonical amino acid (ncAAs) class, which has received considerable attention in recent years [7].

Recently, ncAAs have been utilized for genetic code reprogramming, enabling the incorporation of novel functional elements into proteins. They are also widely applied in the development of biocatalysts with enhanced activity, selectivity, and stability, as well as in the design of new enzyme classes containing artificial regulatory elements, where noncanonical amino acids serve as key catalitic elements [7, 8]. Based on compounds 4 and 5, we synthesized a series of α-alanine derivatives **6**–**9**, differing in the number of carboxyl groups (in Scheme) and having significant potential for structural modification. Existing methods for the synthesis of 3-pyridazinylalanines are often multistep [9] or used an organocatalysts [10].

Experimental part. The solvents were used without prior purification. Starting compounds were kindly supplied by Enamine Ltd. The melting points were determined on a Fisher-Johns apparatus. ^{1}H spectra were measured in DMSO-d_6 solution on a Bruker Avance II 400 (400 MHz on protons). Tetramethylsilane was used as an internal standard. The data were presented as follows: chemical shift, multiplicity (s — singlet, d — doublet, t — triplet, q — quartet, dd — doublet of doublets, m — multiplet), coupling constants (Hz) and integration. HPLC-MS spectra were recorded using the chromatography/mass-spectrometric system consisting of a high-performance liquid chromatograph Agilent 1100, instrument equipped with a diode-matrix and mass-selective detector. The parameters of the chromatography-mass analysis were the column SUPELCO As-

centis Express C18, 2.7 μm 4.6 mm × 15 cm. The elemental analysis was carried out in the Analytical Laboratory of the Institute of Organic Chemistry, NAS of Ukraine. The elemental analysis data corresponded to the calculated data.

Dimethyl 4-(2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)pyridazine-3,6-dicarboxylate (3). In a round-bottom flask, 20 g (0.101 mol) of dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate (**1**), 350 ml of dioxane and 22.93 g (0.101 mol) of methyl 2-((tert-butoxycarbonyl) amino)pent-4-ynoate (**2**) were placed. The resulting reaction mixture was heated to 85 °C and kept at this temperature for 16 h. The solvent was evaporated, and the residue was purified by flash chromatography in the CH_2Cl_2/CH_3OH system with a gradient from 0 to 10 %. Orange powder, yield 34.88 g (yield 86 %). ^{1}H NMR (400 MHz, DMSO-d_6), δ: 1.24 (9H, s), 3.02—3.12 (1H, m), 3.47 (dd, J_1 = 13.8, J_2 = 4.5), 3.65 (3H, s), 3.98 (3H, s), 3.99 (3H, s), 4.44 (1H, (tdd, J_1 = 13.8, J_2 = 4.5, J_3 = 9, Hz), 7.36 (1H, d, J_3 = 9, NH), 8.26 (1H, s).

4-(2-((tert-Butoxycarbonyl)amino)-2-carboxyethyl)pyridazine-3,6-dicarboxylic acid (4). In a round-bottom flask, 34.8 g (0.0868 mol) of triester (**3**), 300 ml of THF and 300 ml of H_2O and 21.89 g (0.521 mol) of LiOH monohydrate were placed. The resulting solution was stirred at room temperature for 16 h. Organic solvent was evaporated, the aqueous layer was transferred to a separating funnel and washed with 3 × 100 ml of methyl-*tert*-butyl ether. The aqueous layer was acidified with a 10 % $NaHSO_4$ solution and extracted with 3 × 200 ml of MTBE. The combined organic phases were dried over Na_2SO_4 and evaporated. Yield 29.58 g (96 %). ^{1}H NMR (400 MHz, DMSO-d_6), δ: 1.24 (9H, s), 2.99 (1H, dd, J_1 = 13.8, J_2 = 9), 3.46 (1H, dd, J_1 = 13.8, J_3 = 4.3), 4.29—4.38 (1H, m), 7.24 (1H, d, J_2 = 9, NH), 8.16 (1H, s).

8-Oxo-5,6,7,8-tetrahydropyrido[3,4-c]pyridazine-3,6-dicarboxylic acid (5). In a round-bottom flask, 2 g (0.0056 mol) of tricarboxylic acid **4** and 40 ml of distilled water were placed. The resulting mixture is heated to 90 °C and kept at this temperature for 6 h. The reaction mixture was evaporated and triturated with THF. A light brown powder, yield 1.6 g (87 %). ^{1}H NMR (400 MHz, DMSO-d_6), δ: 3.34 (1H, d,) 3.33 (1H, d, J_1 = 17), 3.52 (1H, dd, J_1 = 17, J_2 = 6.7), 4.29—4.38 (1H, m), 8.20 (1H, s), 8.69 (1H, d, J = 4.5). LC-MS (EI), m/z: 238 $[M+1]^+$.

4-(2-Amino-2-carboxyethyl)pyridazine-3-carboxylic acid hydrochloride (6). A mixture of 1.6 (0.0067 mol) g pyrido[3,4-*c*]pyridazine **5** and 30 ml concentrated hydrochloric acid was boiled for 16 hours. After cooling to room temperature, the resulting solution was evaporated to dryness under reduced pressure. A light brown powder, yield 1.5 g (90 %). ^{1}H NMR (400 MHz, DMSO-d_6), δ: 3.30—3.41 (2H, m), 4.33—4.44 (1H, m), 8.17—8.22 (1H, d, J = 5), 8.69 (1H, br.s, NH_3^+), 9.39 (1H, d, J = 5).

4-(2-((tert-Butoxycarbonyl)amino)-2-carboxyethyl)pyridazine-3-carboxylic acid (7). 1.5 g (0.006 mol) of compound **6**, 1.28 g (0.015 mol) of $NaHCO_3$, 20 ml of water and 20 ml of THF were placed in a flask, and the resulting solution was cooled to 0 °C. Then, a solution of 1.59 g (0.0073 mol) of Boc_2O in 10 ml of THF was added dropwise to the reaction mixture. The resulting solution was stirred at room temperature for 16 h. After that, the organic solvent was evaporated, the aqueous layer was transferred to a separatory funnel and washed with 3 × 20 ml of MTBE. Then, the aqueous layer was then acidified with 10 % $NaHSO_4$ solution and extracted with 3 × 20 ml MTBE. The combined organic phases were dried over Na_2SO_4 and evaporated. A light brown powder, yield 1.73 g (92 %).^{1}H NMR (400 MHz, DMSO-d_6), δ: 1.27 (9H, s), 2.95 (1H, t, J_1 = 13), 3.22 (1H, dd, J_1 = 13, J_2 = 4.3), 7.20 (1H, d, J = 9), 8.07 (1H, s), 9.27—9.34 (1H, br.s). LC-MS (EI), m/z: 212 $[M+1]^+$.

2-((tert-Butoxycarbonyl)amino)-3-(pyridazin-4-yl)propanoic acid (8). Triacid **4** The solution of 29 g (0.0817 mol) of tricarboxylic acid **4** in 350 ml of dioxane was heated to 95 C and kept at this temperature for 6 h. Then, the solvent was evaporated and the residue crystallized from acetonitrile. A light brown powder, yield 21 g (96 %). ^{1}H NMR (400 MHz, DMSO-d_6), δ: 1.27 (9H, s), 2.84 (1H, dd, J_1 = 14, J_2 = 10), 3.10 (1H, dd, J_1 = 14, J_2 = 4), 4.19—4.25 (1H, m), 7.23 (1H, d, J = 8.5, NH), 7.54 (1H, d, J = 4.5), 9.06—9.14 (2H, m). LC-MS (EI), *m/z*: 268 $[M+1]^+$.

2-Amino-3-(pyridazin-4-yl)propanoic acid dihydrochloride (9). A solution of 20 g (0.0749 mol) of acid **8** in 350 ml of 6N HCl was stirred at room temperature for 4 h and evaporated dryness under reduced pressure. A light brown powder, yield 16.11 g. ^{1}H NMR (400 MHz, DMSO-d_6), δ: 3.31—3.43 (2H, m), 4.38—4.47 (1H, m), 8.08 (1H, d, J = 7, NH), 8.74 (1H, s), 9.41 (2H, m). LC-MS (EI), *m/z*: 168 $[M+1]^+$.

Conclusions. As a result, we have substantiated a convenient preparative low-cost approach to the synthesis of the pyrido[3,4-*c*]pyridazine skeletons and pyridazinyl-α-alanines under IEDDA reaction conditions, the advantages of which are a short reaction pathway without the use of catalysts, simplicity of transformations, and high yields at all stages.

The research was implemented within the framework of the NAS of Ukraine project "Development of new synthetic methodologies based on click chemical reactions and synthesis of non-planar sp^3-enriched bicyclic nitrogen-containing heterocyclic systems with controlled conformational mobility and high pharmacological potential for modern medical and pharmaceutical chemistry", state registration number No. 0121U112165.

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Received 15.07.2025

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РЕАКЦІЯ ДІЛЬСА—АЛЬДЕРА З ОБЕРНЕНИМ ЕЛЕКТРОННИМ ПОПИТОМ ТЕТРАЗИНІВ ТА ФУНКЦІОНАЛІЗОВАНИХ АЛКІНІВ: ЗРУЧНИЙ ШЛЯХ ДО ГЕТЕРОЦИКЛІВ ТА НЕКАНОНІЧНИХ АМІНОКИСЛОТ

У рамках реакції IEDDA (реакції Дільса—Альдера з оберненим електронним попитом) *сим*-тетразину з метил-2-(трет-бутоксикарбоніламіно)пент-4-іноатом розроблено простий препаративний синтез гетероциклічної системи тетрагідропіридо[3,4-c]піридазину. Шляхом його поетапного гідролізу отримано серію піридазиніл-α-аланінів з різною кількістю карбоксильних груп — нових неканонічних амінокислот для біоінженерії.

Ключові слова: *реакція IEDDA, тетразин, 2-амінопент-4-іноат, тетрагідропіридо[3,4-c]піридазин, піридазиніл-α-аланіни, неканонічні амінокислоти.*