ISSN 1070-3632, Russian Journal of General Chemistry, 2010, Vol. 80, No. 12, pp. 2572–2589. © Pleiades Publishing, Ltd., 2010.
Original Russian Text © E.V. Babaev, D.S. Ermolat'ev, 2009, published in Rossiiskii Khimicheskii Zhurnal, 2009, Vol. 53, No. 5, pp. 42–56.

Basic Techniques of Working on a Solid Phase: From ABC of the Peptide Synthesis to Libraries of Non-Natural Amino Acids

E. V. Babaev and D. S. Ermolat'ev

Lomonosov Moscow State University, Vorob'evy Gory 1, Moscow, 119991 Russia
e-mail babaev@org.chem.msu.ru

Received July 10, 2009

Abstract—Libraries of hardly available amino acids bearing a heteroaromatic ring (2-pyrimidyl, substituted 2-pyridyl or 2-thiazolyl) at the amino group were prepared using solid-phase synthesis on various resins. The synthesized compounds are structurally similar to some known antidiabetic drugs. The paper combines features of a review (elementary introduction to the solid-phase synthesis methodology and technique for beginners and selected methods from peptide chemistry) and step-by-step experimental protocols (tested by the authors) useful as a methodic tool. The presented protocols (immobilization and modification of amino acids, placing and removal of common protective groups) require no sophisticated equipment and may be useful as pictorial introductory tasks for students education.

DOI: 10.1134/S107036321012025X

INTRODUCTION

The present paper has a slightly unusual structure. It is based on the experiments aimed at developing a training task for the special student's laboratory course in combinatorial chemistry in the Moscow State University (MSU) and combines features of a review (carefully selected publications on the techniques of peptide synthesis), step-by-step experimental protocols (as procedures tested by the authors), and an original research, specifically, synthesis of a previously inaccessible family of non-natural amino acids with a well-defined structural motif of medicines. In its chemical essence, the chosen reaction sequence is a classical (while slightly reduced) scheme of peptide synthesis:

(1) immobilization of an N-substituted amino acid on a solid support;

(2) removal of the protective group;

(3) modification of the amino acid NH_2 group;

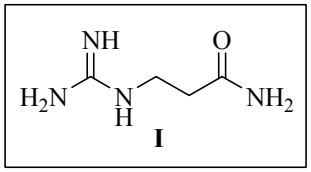
(4) removal of the modified amino acid from the support.

An original element of this task, its peculiar zest, is the last stage in which the amino acid is modified by hetarylation, a reaction fairly rarely used for preparation of non-natural amino acids [1, 2].

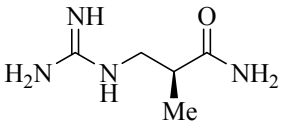
First we substantiate why the molecular structures in the obtained library may suggest biological activity. Then we analyze features of each of the four stages in terms of methodology and published techniques. In conclusion we give concrete practical recommendation and provide experimental protocols, as well as evidence for the structure and purity of the synthesized compounds. The principal methodical feature of the presented protocols consists in that they require no special equipment and can be reproduced in usual student laboratory works.

Problem Statement

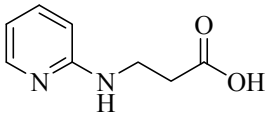
At present the biological activity of 3-guanidinopropionic acid attracts interest of medical chemists due to its expressed antidiabetic activity and, simultaneously, metabolic instability in human's body. Among drugs applied medicines applied, the most known is Tiformin (**I**) which is 3-guanidinopropionamide. Larsen and co-workers [3, 4], optimizing the structure of 3-guanidinopropionic acid and searching for its biologically active analogs, synthesized and tested for biological activity a series of homologs and bioisosteres of this acid:



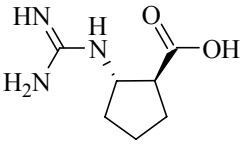
I



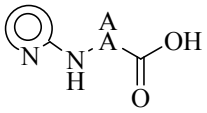
II



III



IV



V

It was shown that activity is largely associated with the amidine fragment of 3-guanidinopropionic acid, whereas point mutations and substitution of the guanidine fragment resulted in partial or complete loss of activity. By contrast, the authors noted that α-alkylation (compound **II**), ring closure in the amidine or guanidine fragment (compound **III**), as well as enhancement of the conformational rigidity of the alkyl chain (compound **IV**) sometimes enhanced the antihyperglycemic activity. It follows from these results that one of the approaches to structural modification of 3-guanidinopropionic acid might involve a systematic screening of heterocycles for replacing the guanidine fragment, as well as the bioisosteric replacement of the carboxy group with wide variation of the structure of the alkyl chain. Thus we can set the task to develop a method of synthesis of a wide series of heterocyclic amino acids of the general formula **V,** having the guanidine and amidine fragments incorporated in an α-aminoheterocycle and an amino acid residue (AA). This class of compounds is well documented, but, even though the problem of preparation of N-hetarylamino acids is quite urgent, no universal synthetic approach to them is still available. Analysis of the literature showed that the principal methods of synthesis of such N-hetarylamino acids is N-alkylation of α-aminoheterocycles and nucleophilic substitutionin the heterocyclic nucleus with amino acid derivatives.

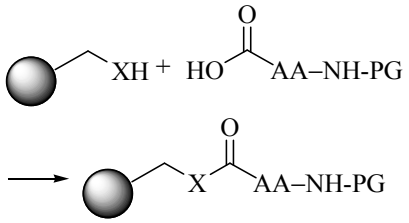
Comparing these two methods, one should take into account that amino acids and their protected derivatives are commercially available, and, consequently, the second method is a more expedient synthetic approach to a wide range of hetarylamino acids. This approach makes use of amino acid derivatives and involves aromatic nucleophilic substitution in heterorings containing a readily leaving group in the α position. It is well known that free amino acids are difficult to apply, since they tend to enter side reactions. Therefore, amino acids should be preliminarily protected and their intermolecular interaction should be minimized.

A radical approach to the problem of synthesis of a series of derivatives **V** by a unified strategy might be provided by solid-phase synthesis. As known, the solid-phase synthetic approach allows, on the one hand, to block the carboxylic function of the amino acid by forming an ester or amide bond with a polymer support and also to exclude the intermolecular interaction of amino acids by forming diketopiperazines and oligopeptides. On the other hand, using a polymer support one can prepared both free acids and esters and amides, which much extends the potential of this method.

As a result, we consider the most acceptable an approach based of the aromatic nucleophilic substitution in heterocycles under the action of amino acids immobilized on a polymer support. (This reaction can also be treated as N-hetarylation of amino acids). As the heterocycles for hetarylation we chose 2-halogenated pyrimidines, 5-nitropyridine, and 5-nitrothiazole. All these halo derivatives fairly readily react with primary and secondary aliphatic amines, the results are thoroughly examined and published.

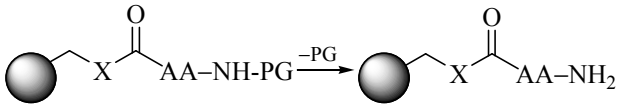
Thus, the chosen strategy for synthesis of N-hetarylamino acids can be represented by the following scheme (here and hereinafter, AA and PP stand for amino acid residue and protective group, respectively).

Stage 1. Immobilization of an N-protected amino acid on a polymer support:

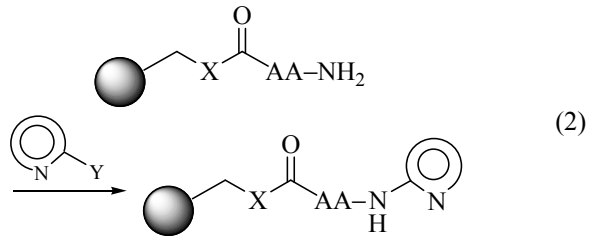


(1)

Stage 2. Deblocking of the amino acid:

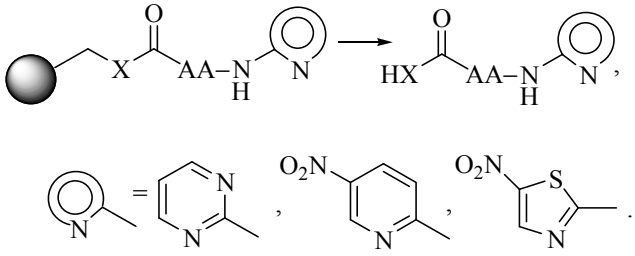


Stage 3. Nucleophilic substitution in the heteroring:



(2)

Stage 4. Removal of the product from the polymer support:



Solid-Phase Organic Synthesis

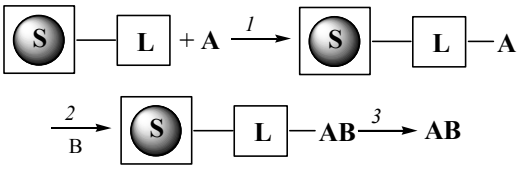
The methodology of modern solid-phase organic synthesis is in many respects adopted from the solid-phase synthesis of peptides. However, there is scarce information in domestic literature on the specific features of solid-phase organic synthesis and the advantages it offers over the traditional liquid-phase synthesis. Therefore, we consider it appropriate to give a brief characteristic of solid-phase synthesis and polymer supports used in this method [5].

The aim of **s**olid-phase organic synthesis is to accelerate the synthetic process due to facilitated isolation and exclusion of additional purification of both intermediate and target products. The term *solid-phase* relates in essence to physical characteristics of a substance on a support, since chemical reaction on a polymer support occurs in a single phase, specifically in a solution. In an appropriate solvent, a polymer swells (Fig. 1) and converts into a low viscous but strongly structured gel (cross-linked polymers) or dissolves (noncross-linked polymers), and synthesis occurs at an ultramicroheterogeneous level, i.e. in an almost homogeneous system.

Fig. 1. Swelling of Merrifield resin in CH_2Cl_2 (as seen, 1 ml of a granular material increases its volume 3 times**)**.

Solid-phase organic synthesis requires the use of a polymer support (resin **S**) and its attached linker **L**. The first stage (stage *1*) involves attachment of a molecule of sub-strate **A** to the linker [5]. Molecule **A** is immobilized but preserves its ability to react with reagent **B** (stage *2*).

Product **AB** stays on the resin, which allows excess reagent **B** (and by-products) to be simply washed out from the product. (Further reagents can be added so that the structure of substrate **A** is successively complicated; the key point is that the linker in these reactions remains untouched). A bifunctional linker **L** is selected so that it is bonded to resin **S** stronger that to substrate **A**. Then at the last stage (stage *3*) the target compound **AB** can be separated from the resin by breaking its bond with the linker. It is clear that the **L**–**AB** bond should be destroyed in mild conditions, not damaging both the compound itself (bond **A**–**B**) and the linker–resin contact (bond **L**–**S**).

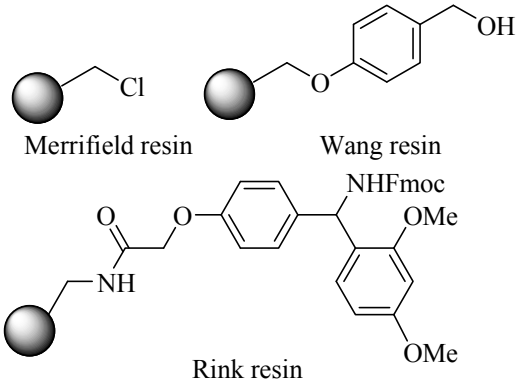


Thus, in an ideal case, by washing the resin after each stage and breaking the bond with the support, one can obtain a pure compound. It is natural to suggest that using a large excess of reagents with subsequent separation from the resin can allow in many cases to shift the chemical equilibrium to the target product and shorten the synthesis time.

The disadvantages of solid-phase organic synthesis include the necessity of using a fairly large excess (2–30 equiv) of reagent, difficulties in the identification of intermediate products, as well as a fairly high cost of modified polymer supports, which depends on the cost of linkers.

Polymer Supports [5]

Chloromethylated polystyrene (cross-linked with a little divinyl benzene) introduced in the practice of organic synthesis by Merrifield (so-called Merrifield resin) is the most accessible of polymer supports.



The chloromethyl group of the polymer allows easy alkylation, say, of carboxylic acid derivatives and their fixation on the resin. However, the resulting ester bond (of the benzyl type) cleaves in fairly rigid conditions (conc. HF, HBr, alkali metal alkoholates, boiling with amines, etc.). In the peptide synthesis, the peptide bond with the Merrifield resin can be cleaved with HF in thioanisole. Such conditions are unfavorable for many compounds, and, therefore, the Merrifield resin is modified to facilitate removal of products from the support. For example, the functional group can be "elongated" by replacing the chlorobenzyl group with a *p*-benzyloxybenzyl (Wang linker) or benzhydryl group (Rink linker).

Such benzyl esters are cleaved with acids in milder conditions. Additional introduction of the electron-donor methoxy groups in the benzene ring further enhances the stability of the benzyl and benzhydryl cations formed by acid hydrolysis of the linker. This allows the Wang linker to be cleaved with 50% CF_3COOH (TFA) and the Rink linker, with 20% TFA. Moreover, modification of the support extends the range of potential reaction products. Thus, with the Wang resin one can obtain acids and alcohols and with the Rink resin, primary and secondary amides.

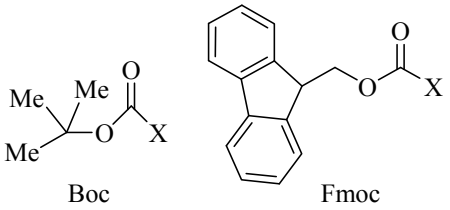
Methodology and Principal Stages of Solid-Phase Peptide Synthesis

The task we set ourselves required a polymer support with a grafted amino acid to be reacted with a heterocycle activated to substitution. Let us consider in more detail the methodical issues of how to prepare amino acids immobilized on polymer supports.

Stage 1. *Immobilization of N-Substituted Amino Acids on Polymer Supports*

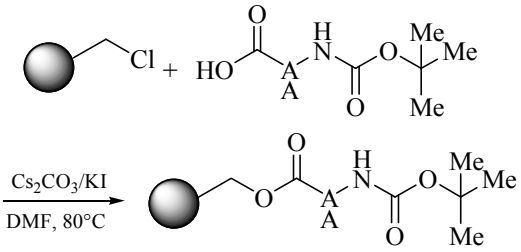
The first stage of our scheme involves immobilization of amino acids on polymer supports (reaction (1)]. To avoid such side processes as oligopeptide formation, amino acids are preliminarily protected. As a rule, N-substituted amino acids are used, and the resulting amino acid–support bond is an amide or ester bond.

In solid-phase organic synthesis, amino groups are most commonly protected by carbamate-type groups, like *tert*-butoxycarbonyl (Boc) [6] and 9*H*-fluorenylmethoxycarbonyl (Fmoc) [7] (X stands for a group to be protected):



It should be noted that the choice of protective group is determined by the type of the polymer support. Immobilization conditions for protected amino acids vary from one polymer support to another.

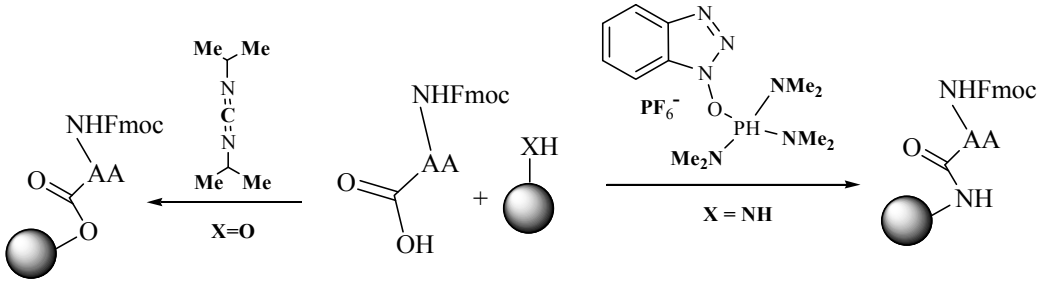
Boc-amino acids are immobilized on the Merrifield resin (chloromethylated polystyrene) *in situ* as cesium salts, by adding a suspension of cesium carbonate in DMF and a potassium iodide catalyst. The reagent-to-support ratio is chosen individually in each case and spans the range 1.5–4.



Immobilization of Fmoc-amino acids on the Wang support (X=O) to form a benzyl ester linker is performed by the carbodiimide method, by treatment with diisopropylcarbodiimide (DIC) in the presence of a 4-(dimethylamino)pyridine (DMAP) catalyst. The immobilization reaction with sterically uncongested amino acids occurs at room temperature. Sterically congested amino acids are immobilized at 40–60°C for 2 days (two immobilization cycles) (Scheme 1).

The immobilization of Fmoc-amino acids on the Rink resin (X=NH) to form a benzhydryl amide linker is performed in the presence of the Castro reagent {(1*H*-1,2,3-benzotriazole-1-yloxy)tris(dimethylamino)-phosphonium (BOP)}, diisopropylethylamine (DIEA) as a base and 1-hydroxybenzotriazole (HOBt) as a catalyst. The reaction occurs at room temperature for

Scheme 1.

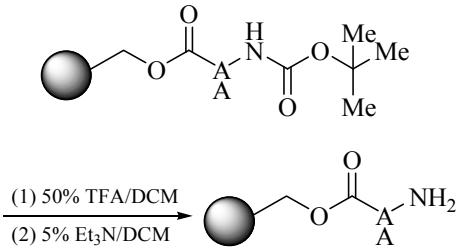


2 or 4–6 h with sterically uncongested and congested amino acids, respectively.

Stage 2. *Deblocking Substituted Amino Acids on Polymer Supports*

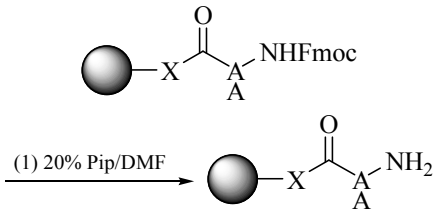
The second stage of our synthetic scheme (after immobilization of a protected amino acid) involves removal of the protective group to activate the amino group. The Boc and Fmoc protections are removed in different ways.

Removal of the Boc protection in amino acids on the Merrifield resin is performed by treatment with 50% trifluoroacetic acid in dichloromethane for 30 min: Under these conditions the Merrifield linker remains intact.



After deprotection the resin is washed with a triethylamine solution to remove trifluoroaaetic acid.

The Fmoc protection in amino acids on the Wang (X=O) and Rink (X=NH) supports is removed with a 20% piperidine solution in DMF for 40–50 min.



A considerable weight loss of the resin after removal of the Fmoc protection can form a basis for gravimetric assessment of the degree of immobilization of protected amino acids at the first stage of solid-phase synthesis.

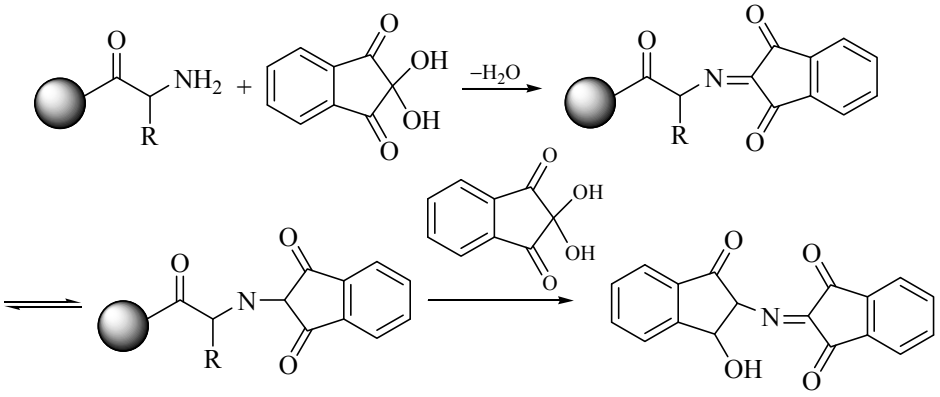
Consecutive treatment of the resin with a solution of piperidine in dimethyl phthalate is recommended: first for 5–10 min and then for 30 min in a fresh solution. After deprotection the resin is washed no less than 4 times with dimethyl phthalate to remove Fmoc decomposition products. The progress of acylation on supports or removal of protective functions from amino acids can be followed by means of the Kaiser test.

Kaiser test for amino group. Analysis of resins after a reaction which results in disappearance or appearance of a free amino group is readily accomplished by means of the ninhydrin (Kaiser) test [8]. This test can be both qualitative and quantitative, and it is a fairly sensitive color reaction for amino group.

A deep dark blue color of the resin and solution provide clear evidence for the presence of a primary amine function on the resin. If the color remains yellow, one can conclude that such function is absent. With secondary (proline) or sterically congested (phenylalanine and β-phenylalanine) amines, the resin and solution acquire a dark red color, which is typical in such cases.

Stage 3. *Nucleophilic Substitution in Heterocycles, Involving Polymer-Immobilized Amino Acids*

The next stage in our planned protocol involves aromatic nucleophilic substitution; the nucleophile is the grafted amino acid and the activated heterocycle is present in solution {reaction (2)}. Most nucleophilic substitutions on supports are accomplished similarly to liquid-phase reactions. However, the reaction temperature should be not above 120°C, since at higher temperatures the polystyrene base of the carrier starts to degrade. Conditions of supported reactions should also preserve the linker.

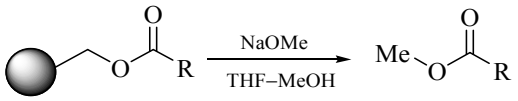


In selecting appropriate activated heterocyclic substrates one should take into account the nature of the leaving group in the heterocycle. Tables 1 and 2 list the activities of pyrimidines with different leaving groups in the 2 and 4 positions.

As seen from Tables 1 and 2, the rate of substitution in 2-halopyrimidines is the highest for the fluorine derivative, the 4 position is more active than the 2 position, and sulfonyl groups are preferred over chlo-rine. The listed data are helpful in selecting other hetarylating substrates.

Stage 4. *Removal of Target Compounds from Polymer Supports [11]*

Most linkers in solid-phase organic synthesis are cleaved in an acid medium. The resistance of linkers to acids is sharply decreased in going from the Merrifield to Wang and Rink resins. The Rink linker is cleaved in milder conditions (10–20% CF_3COOH) than the Wang linker (50% CF_3COOH). The Merrifield resin is passive in such conditions, and it is cleaved by transesterification forming an acid ester [12].



We would like to remind that the nature of the linker determines the type of the terminal function in the molecule removed from the support: The Wang resin allows production of acids, and the Rink resin allows production of amides.

Equipment and Materials

Experimental implementation of the above-described four-stage sequence requires no specific laboratory equipment. Reactions requiring no heating are convenient to perform in capped vials or plastic syringes with a porous partition (Fig. 2a), minimizing manipulations at the stages and filtering and resin washing. If heating is required, glass vials (desirably, from a heat-resistant glass and hermetic screw caps) can be fixed in a temperature-controlled shaker (we used a low-cost domestic device shown in Fig. 2b).

The set of reagents includes three basic components: a "school" set of amino acids, Merrifield and/or Rink resin, and simple substituted heterocycles (vide infra). Commercial immobilized amino acids can be used. In our case (with account for protection/immobilization of amino acids) such reagents as di-*tert*-butyl dicarbonate and 9-fluorenylmethyl chloroformate (introduction of Boc and Fmoc protections) and reagents for

Table 1. Reaction reaction rates of halopyrimidines with piperidine in ethanol

Pyrimidine	Relative reaction rates [9]		
	20°C	30°C	40°C
2-Br	1	2.05	4.1
2-Cl	0.49	1.03	2.11
2-F	66.23	117.7	236
2-I	0.3	0.64	1.35

Table 2. Relative nucleophilic substitution rate constants in activated pyrimidines under the action of cyclohexylamine (29°C, EtOH)

Pyrimidine	Relative rate constant [10]
2-Cl	1.0
2-MeSO-	2.2
2-$MeSO_2$-	4.7
4-MeSO-	1.1×10^3
4-$MeSO_2$-	6.7×10^3
2-$PhSO_2$-	4.5

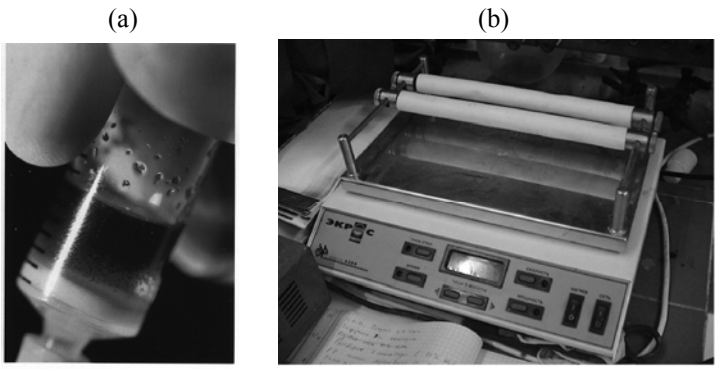


Fig. 2. Equipment for solid-phase synthesis: (a**)** plastic syringe with a porous partition and (b**)** temperature-controlled shaker.

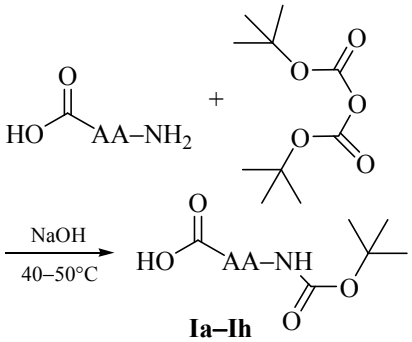
immobilization (Cs_2CO_3/KI, HOBt, or BOP) are needed. Ninhydrin is required for the Kaiser test and CF_3COOH and piperidine for removal of protective groups. At certain stages we took DIEA. A large consumption of solvents, specifically DMF and dichloromethane (DCM), for washing resins should also be taken into account.

PRACTICAL IMPLEMENTATION OF THE TASK

Stage 1: *Immobilization of Amino Acids on Supports*

The starting materials for stage 1 are N-protected amino acids. Boc- and Fmoc-amino acids are commercially available but fairly expensive. By this reason, we considered it reasonable to prepare Boc and Fmoc derivatives of the selected amino acids.

Preparation of Boc-amino acids **I**. Amino acids are heated with di-*tert*-butyl dicarbonate in aqueous dioxane in the presence of NaOH [13].

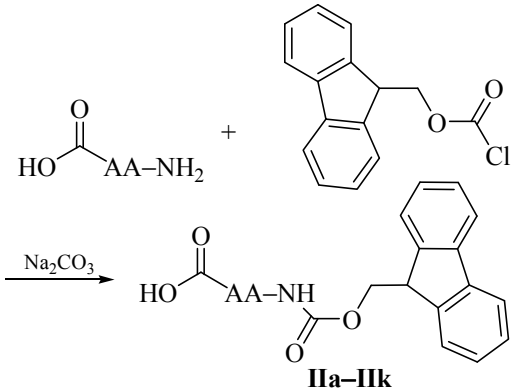


Ia–Ih

Amino acid, 0.04 mol, was added to a solution of 1.6 g of NaOH (0.04 mol) in 40 ml of water. The solution was shaken for 10 min, cooled to 10°C, and a solution of 6.55 g (0.03 mol) di-*tert*-butyl dicarbonate in 30 ml of dioxane was added over the course of 15 min. After 20 min the solution was heated with caution to 40–60°C (CO_2 evolution is initiated) and stirred for 16–40 h until di-*tert*-butyl dicarbonate no longer detected by TLC. The reaction mixture was poured into 100 ml of ice water, and the pH of the solution was brought to 3 by adding 10% HCl in small portions. After chloroform extraction (2×100 ml) the organic phases were combined, and the solution was removed on a rotary evaporator. The remaining colorless oil was crystallized by trituration in hexane in an ultrasonic bath. The products were dried in a vacuum oven and purified, if required, by recrystallization from hexane/ethyl acetate. TLC control: chloroform–methanol–acetic acid 9:1:0.1.

In this way we obtained compounds **Ia–Ih**: Boc-glycine, Boc-L-alanine, Boc-β-alanine, Boc-L-phenylalanine, Boc-β-phenylalanine, Boc-L-proline, Boc-5-aminovaleric acid и Boc-4-(aminomethyl)benzoic acid. The purity of the products was assessed by NMR spectroscopy.

Preparation of Fmoc-amino acids **II**. The Fmoc protection was introduced using 9-fluorenylmethyl chloroformate [7] in the presence of sodium carbonate. By replacing sodium carbonate with DIEA [14] we could decrease the yield of 9-fluorenylmethyl chloroformate hydrolysis products (dibenzofulvene and its polymerization products, as well as 9-fluorenyl-methanol).



IIa–IIk

Method A. Amino acid (20 mmol) was dissolved (or partially suspended), with stirring and cooling on an ice bath, in 50 ml of 10% Na_2CO_3. After that dioxane, 20 ml, was added and then, slowly, a solution of 5.7 g (22 mmol) of 9-fluorenylmethyl chloroformate in 50 ml of dioxane. The mixture was shaken for 1 h at 0°C and then at room temperature from 3 to 8 h, depending on the amino acid. The reaction mixture was diluted to 1 l with ice water and extracted with ether (3×200 ml) to remove chloroformate decomposition products: dibenzofulvene and its polymers (R_f 0.85–0.9; ethyl acetate/hexane 1:1). The aqueous layer was cooled on an ice bath, acidified with conc. HCl to pH 2.0, and extracted with ethyl acetate (4×200 ml). The combined organic phases were washed with 0.1 N HCl and water, dried over Na_2SO_4, and evaporated on a rotary evaporator to obtain a viscous colorless oil which almost always could be crystallized in *n*-heptane on an ultrasound bath.

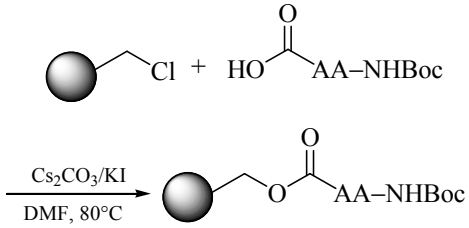
The resulting Fmoc-amino acids were finally purified by recrystallization from ethyl acetate/hexane. TLC control: chloroform/ethanol 3:1 (system I) and chloroform/methanol/acetic acid 9:1:0.1 (system II).

Method B. Here we used DIEA as a base and at least a 20% excess of the base and amino acid with respect to chloroformate, which allowed us to increase the total reaction yield and much decrease by-product formation.

Thus, we could prepare, in fairly high yields, the Fmoc derivatives of glycine, β-alanine, 4-aminobutyric acid, 5-aminovaleric acid, L-alanine, L-phenylalanine, β-phenylalanine, L-asparagine, DL-valine, L-proline, and 4-(aminomethyl)benzoic acid. The resulting products were characterized and their purity was assessed by NMR spectroscopy.

Immobilization of Boc- and Fmoc-protected amino acids was performed by standard procedures.

Boc-substituted amino acids were immobilized on the Merrifield acid in the presence of cesium carbonate and a potassium iodide catalyst at 80–85°C:

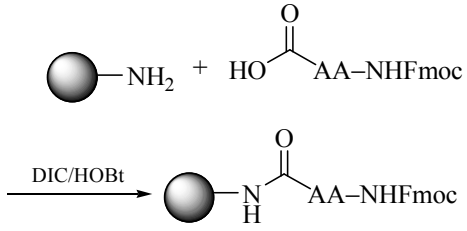


The Merrifield resin (1 g, 1.6 mmol) was placed in a 30-ml vial, poured with 3 ml of DMF, and left to swell for 10–15 min. A solution containing **V** mmol of a Boc-amino acid, 1.6 g (5 mmol) of Cs_2CO_3, and a catalytic amount of NaI (1 mmol, 0.17 g) was then added to the swollen polymer so that the total volume of the reaction mixture was no more than 10 ml. Tightly capped vials were heated in an oil bath at 80–85°C for 12 h with intermittent shaking. The resin was then separated on a glass frit, washed successively with DMF (20 ml), 10% NH_4Cl (3×30 ml), DMF (20 ml), DCM (2×30 ml), and MeOH (2×15 ml), dried a in vacuum oven, and weighed.

The yields at the stage of immobilization of protected amino acids on the Merrifield resin were as follows, %:

Glycine (Gly)	94
β-Alanine (β-Ala)	95
5-Aminovaleric (5-Ava)	92
L-Alanine (L-Ala)	96
L-Phenylalanine (L-Phe)	82
DL-β-Phenylalanine (DL-β-Phe)	76
L-Proline (L-Pro)	95
4-(Aminomethyl)benzoic (Amb)	97

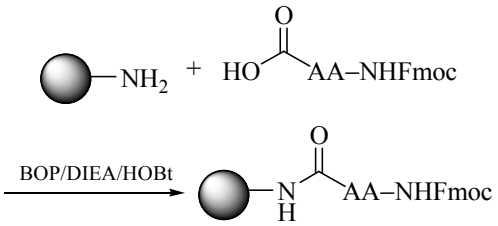
The Fmoc-substituted amino acids were immobilized on the Rink resin by treatment with DIC in the presence of 1-hydroxybenzotriazole:



Carbodiimide method. A dry Rink-NH_2 resin (1 g) was placed in a 30-ml vial, poured with 3 ml of a solution of 0.46 g (3 mmol) of 1-hydroxy-1*H*-1,2,3-benzotriazole in 1:1 DMF/DCM, and left to swell for 10 min. After that 3–4 of a 1:1 ml DMF/DCM solution containing 3 mmol of a *Fmoc*-amino acid and 3 mmol (0.38 g) of DIC. If a precipitate formed, more DMF was added. Tightly capped vials were agitated on an orbital shaker for 15–30 h. The reaction progress was followed by the Kaiser test. As a rule, the reaction time was 4–5 h with unbranched amino acids and 6–9 h with sterically congested amino acids. After reaction

completion the resin was washed in succession with DMF (3×20 ml), DCM (2×20 ml), and MeOH (2×10 ml) and dried in a vacuum oven. The resin weight gain always nicely correlated was the calculated reaction yield.

Sterically congensted amino acids, such as L-phenylalanine, L-asparagine, DL-valine, and β-phenylalanine were immobilized on the Rink resin by means of (1*H*-1,2,3-benzotriazol-1-yloxy)tris (dimethylamino)-phosphonium hexafluorophosphate (BOP) and diisopropylethylamine (DIEA) in the presence of 1-hydroxybenzotriazole (HOBt).



A solution (6–7 ml) of 1.33 g (3 mmol) of BOP, 3 mmol Fmoc-amino acid, and 0.78 g (6 mmol) of DIEA in 1:1 DMF/DCM was added to deblocked Rink resin (1 g). The mixture was agitated on an orbital shaker for 5 h. The resin was separated, washed with DMF (2×20 ml), DCM (2×30 ml), and MeOH (1× 15 ml), and dried in a vacuum oven. The reaction progress was followed by the Kaiser test.

The yields at the stage of immobilization of protected amino acids on the Rink resin were as follows, %:

Glycine (Gly)	99
β-Alanine (β-Ala)	100
4-Aminobutyric (GABA)	98
5-Aminovaleric (5-Ava)	99
L-Alanine (L-Ala)	98
L-Phenylalanine (L-Phe)	89
DL-β-Phenylalanine (DL-β-Phe)	94
DL-Valine (DL-Val)	90
L-Proline (L-Pro)	98
L-Asparagine (L-Asp)	87
4-(Aminomethyl)benzoic (Amb)	95

In total, we obtained at the first stage 19 supports (1–4 g) containing N-substituted amino acids: 8 amino acids on the Merrifield resin and 11 amino acids on the Rink resin.

The yields at the immobilization stage were determined by gravimetry. As a rule, the carbodiimide method results in almost quantitative immobilization yields on the Rink resin. With sterically congested amino acids, the yields are slightly lower.

Determination of the immobilization degree of Fmoc-amino acids. To a resin obtained by coupling 1 g of the Rink-NH_2 resin with Fmoc-amino acids in standard conditions, 14 ml of a 25% solution of piperidine in DMF was added. The vial was shaken for 40–45 min, and the resin was separated on a glass filter and washed with DMF (3×10 ml). The mother liquor was diluted with ice water to 250 ml. 1-(9*H*-Fluoren-9-ylmethyl)piperidine which precipitated as white flakes was filtered off, washed with water, and dried in a vacuum, mp 119–120°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 7.77 m (H_{16}, H_{20}, 2H), 7.33 d (H_{13}, H_{17}, 2H), 7.15 m (H_{14}, H_{15} H_{18}, H_{19}, 4H), 5.21 t (CH, J = 5.5 Hz, 1H), 3.71 d (CH_2, J = 5.15 Hz, 2H), 2.50 m ($2CH_2$, 4H), 1.68 m ($3CH_2$, 6H). The yield of the immobilization reaction of Fmoc-amino acids on the Rink resin was calculated by the formula:

$$\text{Yield} = \frac{m(\text{precipitate})\times 1000}{MC}\times 100\%, \qquad (3)$$

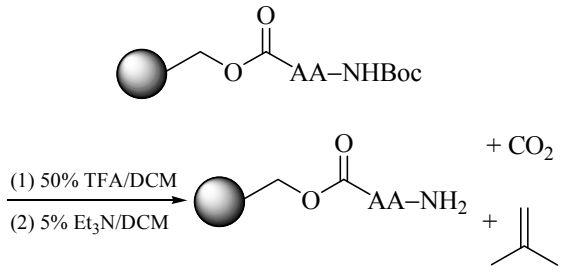
where m is the weight of the precipitate, M = 263.4, molecular weight of 1-(9*H*-fluoren-9-ylmethyl)piperidine, and C = 0.73, capacity of the Rink resin, mmol/g.

Stage 2: *Removal of Protective Groups*

The stage of immobilization is followed by the stage of protection removal. Free amino groups on the resin are readily determined by the Kaiser test (vide supra).

Kaiser test. In 3 vials we prepared by 50 ml of the following solutions: ~80% of phenol in ethanol; a mixture of water and pyridine (1:4), containing 1 ml of 002 M NaCN; and 6% of ninhydrin in ethanol. A little resin from the reaction vessel was transferred on a Schott microfilter (0.5 ml), the resin was washed with three portions of MeOH, transferred to a 3-ml tube, each of the above solution (by 3 drops) was added, and the tube was heated at 120°C with shaking for 3–5 min.

The Boc protection was removed by treatment with 50% TFA for 0.5 h. During reaction the resin suspension foams because of vigorous gas evolution:



Removal of the Boc protection from amino acids on the Merrifield resin. A 55% solution of TFA in DCM (20 ml) was poured onto a 1-g sample of the resin with an immobilized amino acid placed in a high 30-ml vial. Gas evolution was observed, and the resin acquired a characteristic pinkish color. The vial was shaken for 0.5 h, and the resin was separated on a glass filter and washed with DCM (2×20 ml), 10% DIEA in DCM (2×30 ml), DMF (2×10 ml), DCM (15 ml), and MeOH (15 ml). The resin was dried in a vacuum oven and weighed. Amino groups were detected by the Kaiser test.

The Fmoc protection from amino acids immobilized on the Rink support were selectively removed with a 20% solution of piperidine in DMF. The intermediate dibenzofulvene (DBF) rapidly reacts with excess piperidine to form 1-(9*H*-fluoren-9-ylmethyl)-piperidine which is water-insoluble and precipitates when the reaction mixture is diluted with water (Scheme 2).

With the weight of a dried 1-(9*H*-fluoren-9-ylmethyl)piperidine precipitate, the yield of immobilization of Fmoc-amino acids on the Rink resin is calculated by formula (3).

The yields of immobilization of Fmoc-amino acids on the Rink resin were as follows, %:

Glycine (Gly)	89
β-Alanine (β-Ala)	90
5-Aminovaleric (5-Ava)	84
L-Alanine (L-Ala)	94
L-Phenylalanine (L-Phe)	82
L-Proline (L-Pro)	86

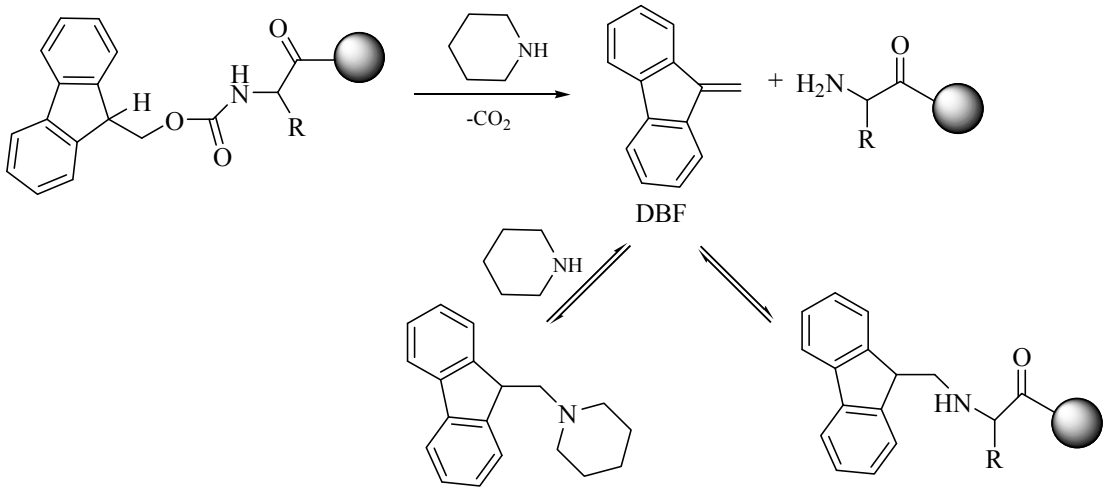
Removal of the Fmoc protection from amino groups on resins. A 20% solution of piperidine in DMF (20 ml) was poured on a resin containing a Fmoc-protected amino group or Fmoc-protected amino acid, placed in a 30-ml standard vial with a porous bottom. The resin was separated from the solution, and the procedure was repeated; therewith, additional 30-min shaking was applied. The resin was washed in succession with DMF (3×30 ml), DCM (2×25 ml), and MeOH. (2×15 ml). Amino groups were detected by the Kaiser test.

Synthesis of Heterocycles with a Readily Leaving Group

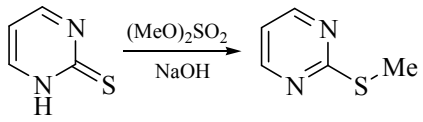
Experiments on hetarylation of amino acids were performed with four fairly active heterocycles: 2-(methylsulfonyl)pyrimidine (**IV**), 2-bromo-5-nitro-1,3-thiazole (**V**), 2-fluoropyrimidine (**VI**), and 2-chloro-5-nitropyridine (**VII**).

Pyrimidine **IV** was prepared in a moderate yield by oxidation of 2-(methylsulfanyl)pyrimidine (**III**) with *m*-chloroperbenzoic acid [15]. The starting pyrimidine **III** is readily prepared by methylation of commercial

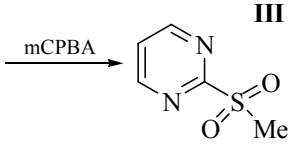
Scheme 2.



pyrimidine-2-thione with dimethyl sulfate in the presence of NaOH [16].



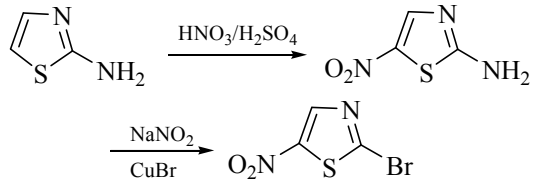
III



IV

A solution of 32 g (0.13 mol) of 3-chloroperbenzoic acid in 300 ml of chloroform was added dropwise with cooling (–25°C) and vigorous stirring over the course of 1.5 h to a solution of 8.2 (0.065 mol) of compound **III** in 100 ml of chloroform. The mixture was stirred at 20°C for a day. After the reaction had been complete, the reaction mixture was washed with a saturated solution of sodium sulfite (2×300 ml) and then with a saturated solution of sodium hydrocarbonate (2×400 ml). The combined organic phases were dried over sodium sulfate. The solvents were distilled off, the dry residue was recrystallized from ethanol to obtain, after drying, 4.75 g (46%) of compound **4** as colorless crystals, mp 73–75°C. TLC control: R_f 0.6 (methanol–chloroform, 1:9. ^{1}H NMR spectrum (360 MHz, $CDCl_3$) δ, ppm: 8.94 d (2CH, J = 4.3 Hz, 2H); 7.58 t (CH, J = 5.6Hz, 1H); 3.36 s (CH_3, 3H).

2-Bromo-5-nitro-1,3-thiazole (**V**) was prepared by nitration of 2-amino-1,3-thiazole followed by diazotization and introduction of bromine in position 2 by the Sandmeyer reaction [17]:

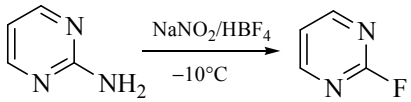


V

2-Amino-1,3-thiazole, 20 g (0.2 mol), was added with caution, in small portions, over the course of 0.5 h, to 55 ml of a cold (to –5°C) conc. H_2SO_4 placed in a 0.5-l high glass. The mixture was then cooled to –10°C, and 10 ml of fuming nitric acid (0.23 mol) was added dropwise over the course of 25 min under vigorous stirring. The solution was left to stand for three days at room temperature in an uncovered glass. The bright orange mixture was poured into 150 ml of ice water, and a solution of 100 g of NaBr and 100 g of $CuSO_4$ in 200 ml of water was added to it. The mixture was cooled to –3°C and diazotized with a solution of 25 g of sodium nitrite in 35 ml of water under vigorous stirring for 1 h, after which it was diluted with water to 500 ml and extracted with ether (3×200 ml). The combined organic phases were dried over calcined Na_2SO_4. The ether was removed on a rotary evaporated, and the residue was subjected to steam distillation. After drying, 15.89 g (38%) of compound **V** was obtained as light weight crystals. TLC control: R_f 0.51 (hexane–ethyl acetate, 5:1). mp 88–91°C. ^{1}H NMR spectrum (360 MHz, $CDCl_3$), δ, ppm: 8.30 s (CH, 1H).

It should be added that the ^{1}H NMR spectrum is poorly informative and contains only one signal at δ 8.3 ppm. The IR spectrum (KBr) is more informative: 3084 v (C–H), 1520 as, 1344 s (NO_2), 740 (C–Br). For comparison, the IR spectrum of compound **V** from the site of Acros (www.acros.com) can be used.

2-Fluoropyrimidine (**VI**) was prepared by diazotization of 2-aminopyrimidine under the action of sodium nitrite in 48% HBF_4 [10]. 2-Fluoropyrimidine is so reactive that on attempted neutralization of the reaction mixture with 1N NaOH by the procedure [10] we always obtained a hydrolysis product, i.e. pyrimidin-2-one. It was found that the yield of compound **VI** can be improved by using a suspension of sodium hydrocarbonate instead of a solution of NaOH and cooling (to –10°C) during neutralization.



VI

A solution of 15.3 g of 2-aminopyrimidine (0.16 mol) in 500 ml of 48% HBF_4 was poured into a high 1-l porcelain glass, cooled to –12ºC, and subjected to diazotization under vigorous stirring by treatment with a solution of 22.8 g of sodium nitrite (0.32 mol) in 35 ml of water for 1 h. After adding sodium nitrite, the mixture was stirred for an additional 1 h at –10ºC and then neutralized with a saturated solution of sodium hydrocarbonate to pH 6–7 under cooling and extracted with ether (4×250 ml). The combined organic layers were washed with a 2% solution of potassium carbonate (2×100 ml) and dried over calcinated Na_2SO_4. The solvent was distilled off at the water bath temperature of no higher than 20ºC. The oily residue was distilled in a vacuum to obtain 6.4 g (41%) of a transparent mobile liquid, bp 72–73ºC (20 mm). The product can be stored at 0ºC with no appreciable decomposition signs for 10 days. TLC control: R_f 0.61 (ethyl acetate, 1:1). ^{1}H NMR spectrum

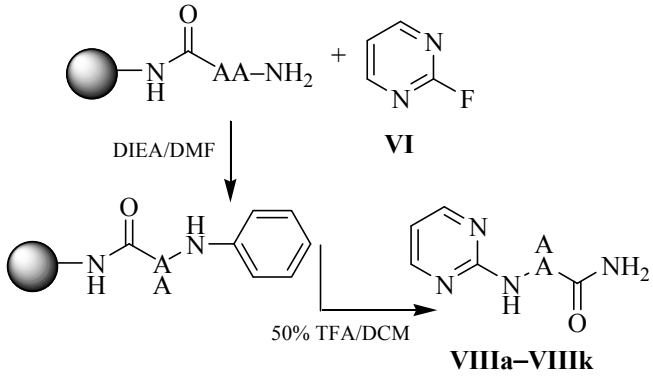
(360 MHz, $CDCl_3$) δ, ppm: 8.66 q (2CH, $J_{C4(6),5}$ 6 Hz, $J_{C4,F}$ 1.7, 2H); 7.38 q (CH, $J_{C4,5}$ 6 Hz, $J_{C5,F}$ 1.7, 1H).

2-Chloro-5-nitropyridine **VII**. A commercial reagent was used. (Synthesis of compound **VII** is described in detail in [18].)

Realization of Stage 3 (Solid-Phase Nucleophilic Substitution) and Stage 4 (Product Removal from Polymer Support)

Synthesis of N-(pyrimidin-2-yl)amino Acid Derivatives

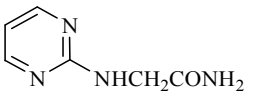
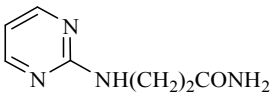
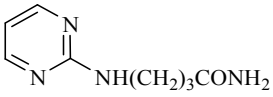
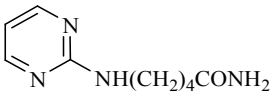
The nucleophilic substitution reactions with amino acids immobilized on the Rink resin were performed using 2-fluoropyrimidine (**VI**) and DIEA as the base. During reaction the resin volume decreased 1.5–2 times, which, too, was evidence for reaction progress. For control of reaction progress we used the Kaiser test for amino group, taking from the reaction mixture small resin portions.



The resulting primary amides of *N*-(pyrimidin-2-yl)-amino acids were removed from the support by treatment with 50% TFA. Compounds **VIIIa**–**VIIIk** were purified by column chromatography. Structural assessment was performed by ^1H NMR spectroscopy and mass spectrometry. The elemental analyses and ^1H NMR spectral data are listed in Tables 3 and 4.

Synthesis of amides of N-(pyrimidin-2-yl)amino acids. A solution of 0.52 (4 mmol) of DIEA in absolute DMF was poured onto 0.5 g of the Rink resin containing an unsubstituted amino acid. After 7–10 min, when the resin gained weight, 0.39 g (4 mmol) of 2-fluoropyrimidine was added. The viald were tightly closed and heated at 50–55°C with intermittent shaking. The Kaiser test showed that the reaction was almost complete in 7–12 h. The reacted resin was successively washed on a filter with DMF (15 ml), DMF/DCM (15 ml 50%), DCM (2×25 ml), and MeOH (2×15 ml) and dried in a vacuum oven, after which it was transferred into a 30-ml flask, poured with 15 ml of 60% TFA/DCM, and agitated on an orbital shaker for 1 h. The resin was separated on a glass filter and washed with absolute DCM (15 ml) and MeOH (15 ml). The filtrate was evaporated on a rotary evaporator. The oily residue was poured with absolute MeOH (5 ml) and evaporated again. The procedure was repeated until TFA was removed completely. The product was purified, if necessary, by column chromatography (MeOH–$CHCl_3$, 4:1) on silica. TLC was performed in MeOH–$CHCl_3$, 4:1. The yield (Table 4) was calculated on the basis of the standard resin capacity.

Table 3. Elemental analyses of amides **VIII**

Compound		Element content, %		
		C	N	
	Theor.	5.26	47.32	36.84
	Exp.	5.74	46.88	36.57
	Theor.	6.02	50.54	33.69
	Exp.	6.73	49.48	32.6
	Theor.	6.66	53.27	31.07
	Exp.	6.38	52.51	30.95
	Theor.	7.2	55.6	29.86
	Exp.	6.89	53.75	29.32

For the activated pyrimidine for the reaction of activated pyrimidines with amino acids on the Merrifield resine we chose 2-(methylsulfonyl)pyrimidine (**IV**):

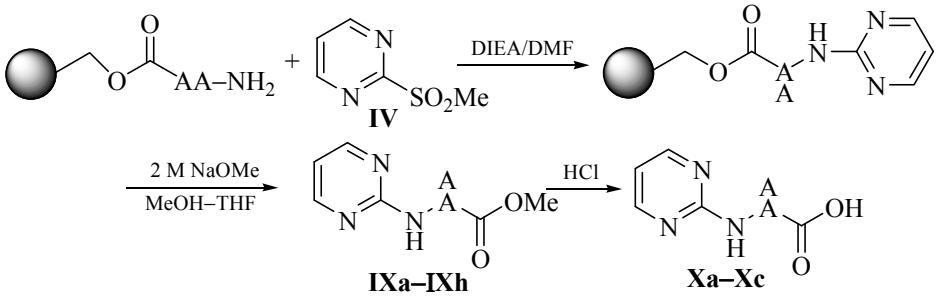
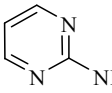
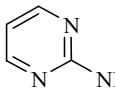
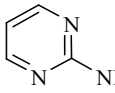
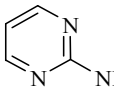
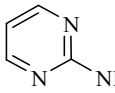
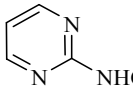
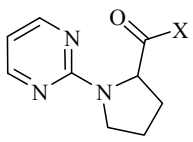
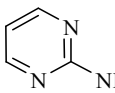


Table 4. Yields and chatacteristics of amide derivatives and *N*-(pyrimidin-2-yl)amino acid esters (^{1}H NMR at 36-MHz, DMSO-d_6)

Comp. no.	Compound	X	^{1}H NMR spectrum, δ, ppm			Mass spectrum M^+, *m/z*	Yield, %
			$H_{4,6}$	H_5	NH		
IXa		MeO	8.22	6.55	7.14	167	85
VIIIa		NH_2	8.32	6.54	7.09	152	96
IXb		MeO	8.19	6.46	6.75	181	94
VIIIb		NH_2	8.31	6.63	7.31	166	98
VIIIc		NH_2	8.36	6.75	6.64	180	87
IXc		MeO	8.26	6.53	6.85	209	34
VIIId		NH_2	8.28	6.56	6.47	194	65
IXd		MeO	8.22	6.54	7.14	182	79
VIIIe		NH_2	8.33	6.54	7.25	166	88
IXe		MeO	8.25	6.53	6.66	217	32
VIIIf		NH_2	8.20	6.53	7.45	242	49
IXf		MeO	8.31	6.68	6.58	218	11
VIIIg		NH_2	8.19	6.48	6.58	242	23
VIIIh		NH_2	8.34	6.74	7.27	209	28
VIIIi		NH_2	8.25	6.57	6.81	195	35
IXg		MeO	8.25	6.71	–	208	52
VIIIj		NH_2	8.27	6.57	–	192	72
IXh		MeO	8.48	6.81	7.46	244	74
VIIIk		NH_2	8.18	6.59	7.07	228	83

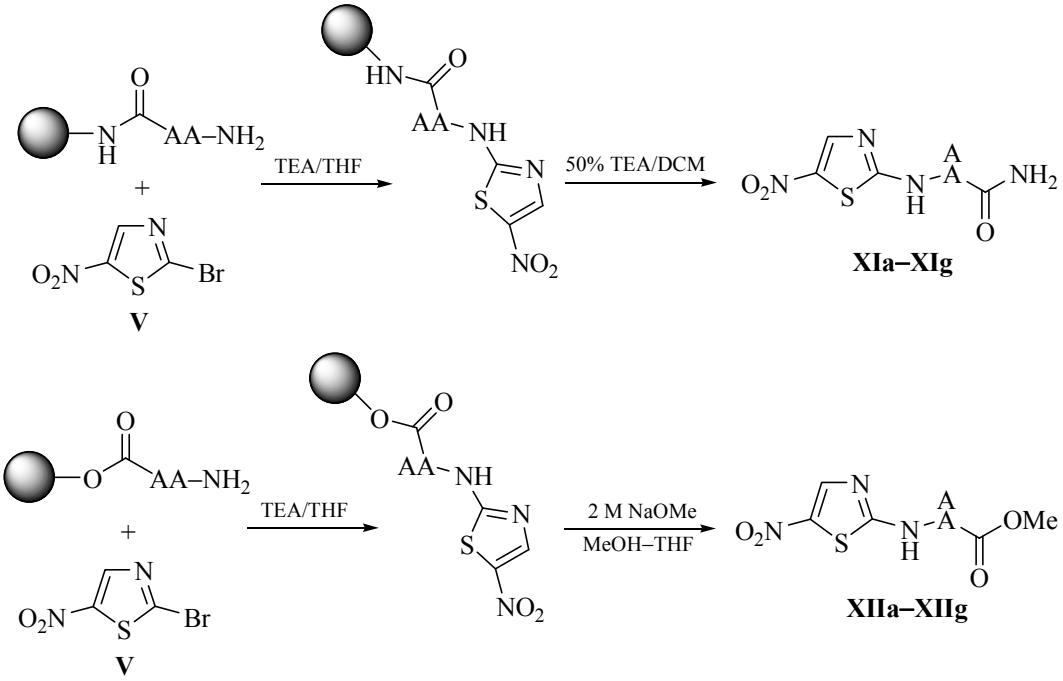
Reaction progress was followed by the Kaiser test for amino group. Removal of products from the resin was performed in the presence of sodium methylate. The resulting *N*-(pyrimidin-2-yl)amino acid esters **IXa–IXh** were characterized by the mass and ^{1}H NMR spectra (Table 4). Three examples were used to demonstrate the possibility to prepare free amino acids, viz. *N*-(pyrimidin-2-yl)amino acids **Xa–Xc,** by hydrolysis of the corresponding esters.

Synthesis of methyl esters of N-(pyrimidin-2-yl) amino acids **(X)**. A solution (6 ml) of 0.65 (5 mmol) of DIEA in absolute DMF was poured onto 1 g of the Merrifield resin containing an unprotected amino acid. After **V** min, 0.79 g (5 mmol) of pyrimidine **IV** was added to the resin. The vials were tightly closed and heated at 80–90°C with intermittent shaking. Reaction progress was followed by the Kaiser test. The reacted resin was successively washed on a filter with DMF (25 ml), DCM (2×25 ml), and MeOH (2×15 ml), after which it was transferred to a standard 30-ml vial and poured with absolute THF (3 ml) and 2M NaOMe in MeOH (1 ml). The vials were tightly closed and shaken at room temperature for 6 h. The resin was separated on a glass filter and washed with MeOH and THF. The filtrate was neutralized with 10% HCl and evaporated on a rotary evaporator. The dry residue was treated with methanol to extract the resulting ester, the extract was filtered off, and evaporated on a rotary evaporator. The product is purified, if required, by column chromatography (MeOH–$CHCl_3$, 2:9) on silica. The yield (Table 4) was calculated on the basis of the resin capacity. TLC was performed in MeOH–$CHCl_3$, 2:9.

Synthesis of amino acids **X** *from esters* **IX**. Ester **IX** (50–200 mg) was dissolved in 5 ml of 40% aqueous MeOH, after which 1 ml of conc. HCl was added. The solution was heated at 60°C for 10–15 min and evaporated. The acid was recrystallized was aqueous methanol. TLC was performed in MeOH–$CHCl_3$–AcOH, 1:3:0.2. Acids **X** were prepared on the scale of 30–150 mg.

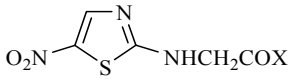
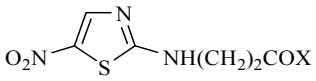
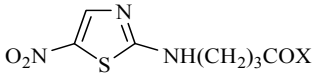
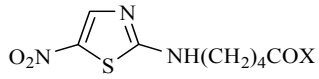
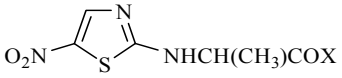
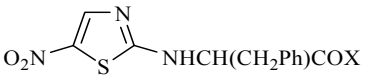
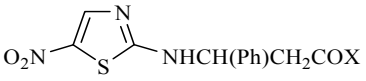
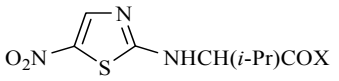
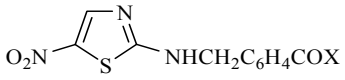
Synthesis of N-(5-nitrothiazol-2-yl)amino Acid Derivatives

Amino acids immobilized on the Rink and Merrifield resins were reacted with 2-bromo-5-nitrothiazole **V** in the presence of triethylamine:



Synthesis of N-(5-nitrothiazol-2-yl)amino acid amides. A solution (4 ml) of 0.2 (2 mmol) of triethylamine in absolute DMF was added to 0.5 g of the Rink resin containing an unprotected amino acid. After 10 min, 0.42 g (2 mmol) of 2-bromo-5-nitrothiazole was added. The vial was tightly closed and cooled to 5°C with constant shaking. The Kaiser test showed that the reaction is almost complete in 1–2 h. The reacted resin was successively washed on the filter with DMF (4× 10 ml), DCM (2×15 ml), and MeOH (2×15 ml). After

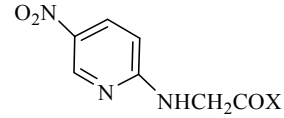
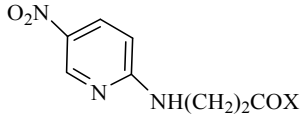
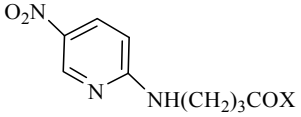
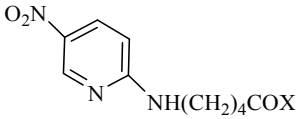
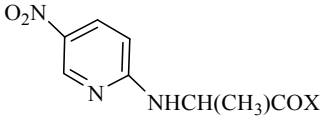
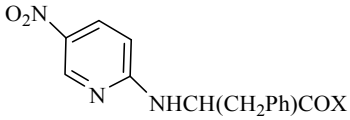
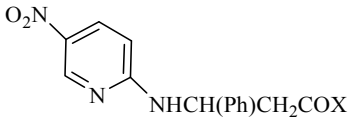
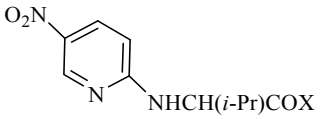
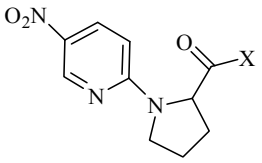
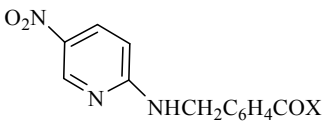
Table 5. Yields and characteristics of *N*-(5-nitrothiazol-2-yl)amino acid amides **XI** and esters **XII**

Comp. no.	Compound	X	^{1}H NMR spectrum, δ, ppm		Mass-spectrum $[M^+ - NO_2]$, *m/z*	Yield, %
			H_4	NH		
XIIa		MeO	8.07	7.25	172	81
XIa		NH_2	8.09	7.53	157	85
XIIb		MeO	7.95	7.32	186	89
XIb		NH_2	7.94	7.56	171	76
XIc		NH_2	8.XII	7.37	184	79
XIIc		MeO	7.91	7.34	214	92
		NH_2	–	–	198	–
XIId		MeO	7.99	7.41	186	84
XId		NH_2	8.07	7.45	171	88
XIIe		MeO	7.90	–	262	38
XIe		NH_2	8.08	7.82	247	27
XIIf		MeO	7.92	–	262	52
—		NH_2	–	–	248	—
XIf		NH_2	8.01	7.89	185	22
XIIg		MeO	7.95	7.37	248	64
XIg		NH_2	8.11	7.65	233	55

drying in a vacuum oven and placed in a 30-ml vial, poured with 55% TFA/DCM (10 ml), and agitated on an orbital shaker for 1 h. The resin was separated on a glass filter, washed with absolute DCM (5 ml) and absolute MeOH (5 ml). The filtrate was evaporated on a rotary evaporator. The oily residue was poured with absolute MeOH (5 ml) and evaporated again. The procedure was repeated until TFA was removed completely. The product was purified, if necessary, by column chromatography (MeOH–$CHCl_3$, 1:2) on silica. The yield was calculated on the basis of the average resin capacity. Thus we obtained amides **XIa**–**XIe, XIf,** and **XIg (**50–150 mg).

Synthesis of methyl esters of N-(5-nitrothiazol-2-yl) amino acids. A solution (3 ml) of 0.3 g (3 mmol) of triethylamine in absolute THF was added to 0.5 g of the Merrifield resin containing immobilized unprotected amino acids. After 10 min, a solution (3 ml) of 0.65 g (3 mmol) of 2-bromo-5-nitrothiazole in THF was added to the suspended resin. The reaction was performed in cold with intermittent shaking. The resin got intense red. After 10 h, the resin was separated on a glass filter, washed with DMF (2×20 ml), DCM (2×30 ml), and MeOH (2×15 ml), dried in a vacuum oven, and weighed. The dry resin was placed in a standard 30-ml vial and poured with absolute THF (3 ml) and 2M NaOMe in MeOH (1 ml). The vial was tightly closed and shaken at room temperature for 6–12 h. The resin was separated on a glass filter and washed with 30% MeOH in THF. The filtrate was neutralized with 10% HCl and evaporated on a rotary evaporator. The dry residue was treated with methanol to extract the resulting ester, and the extract was evaporated on a rotary

Table 6. [1]H NMR data (360 MHz, DMSO-d_6) and characteristics of *N*-(5-nitropyridin-2-yl)amino acid derivatives

Comp. no.	Formula	X	[1]H NMR spectrum, δ, ppm			Purity[a]	Yield, %
			H_6	H_4	H_3		
XIVa		MeO	8.87	8.15	6.53	B	57
		NH_2	8.87	8.16	6.77	B	72
XIVb		MeO	8.84	7.96	6.57	C	68
		NH_2	8.82	7.98	6.55	C	60
XIIIc		NH_2	8.83	7.98	6.51	C	57
XIVc		MeO	8.82	8.09	6.61	C	70
		NH_2	8.83	7.97	6.54	C	47
XIVd		MeO	8.80	8.14	6.65	B	52
		NH_2	8.83	7.97	6.62	C	84
–		MeO	–	–	–	–	–
		NH_2	8.80	8.16	6.67	B	28
–		MeO	–	–	–	–	–
		NH_2	8.81	7.97	6.58	C	18
XIIIh		NH_2	8.83	8.03	6.81	B	12
–		MeO	–	–	–	–	–
		NH_2	8.88	8.12	6.78	–	32
XIVe		MeO	8.85	8.14	6.55	A	69
		NH_2	8.84	8.02	6.59	A	39

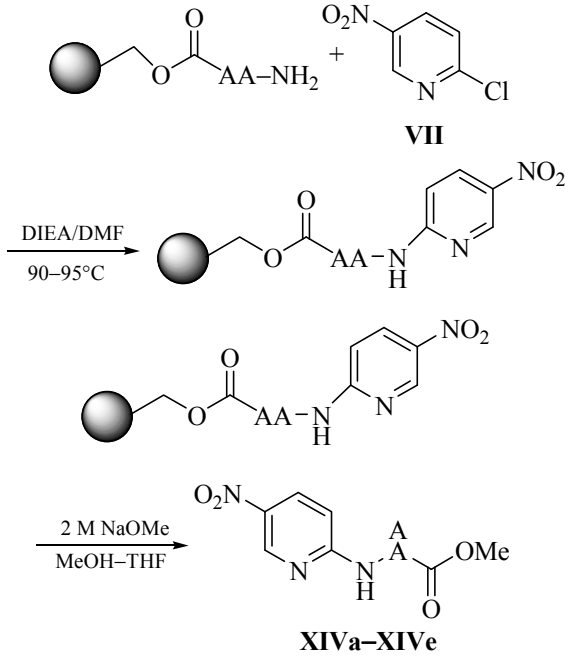
[a] The purity of products **XIIIa–XIIIg** and **XIVa–XIVe** was estimated by the [1]H NMR spectra as follows: A (<5% of admixtures), B (<10% of admixtures), and C(>20% of admixtures).

evaporator. The product was recrystallized from hexane–ethyl acetate, 3:1, and dried in a vacuum oven. Thus we obtained esters **XIIa**–**XIIg (**20–170 mg).

With both the resins, an intense yellowish red coloration of the resin was observed. The Kaiser test showed that the reaction with 2-bromo-5-nitrothiazole occurs at room temperature for 3–6 h.

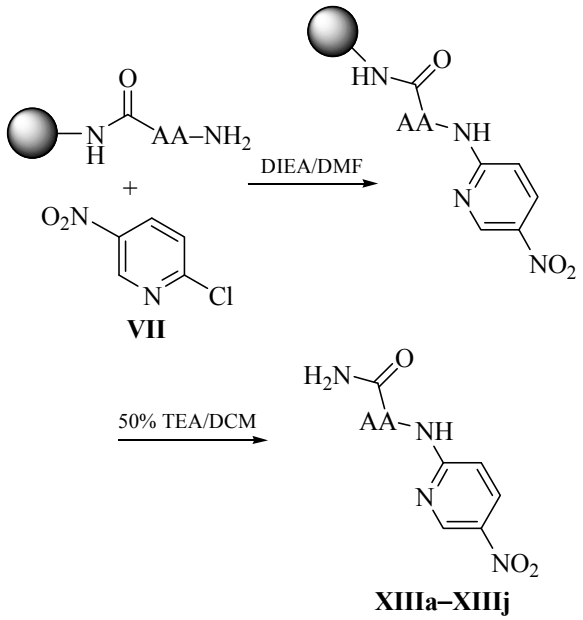
The resulting primary amides and methyl esters of *N*-(thiazol-2-yl)amino acids were characterized the mass and ^{1}H NMR spectra. The characteristics of compounds **XIa–XIg** and **XIIa–XIIg** are listed in Table 5.

The yields of chromatographic purity of the obtained *N*-(thiazol-2-yl)amino acid derivatives proved to be lower than those of *N*-(pyrimidin-2-yl)amino acid derivatives. We can suggest that, along the main nucleophilic substitution reaction, 2-bromo-5-nitrothiazole undergoes the side reaction involving thiazole ring opening, which was mentioned in [19]:



Synthesis of N-(5-nitropyridin-2-yl)amino Acid Derivatives

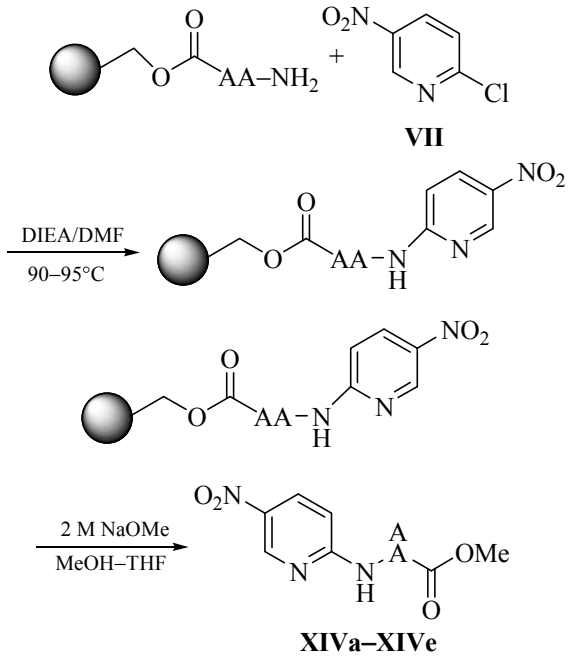
N-(Pyridin-2-yl)amino acid derivatives on the Rink resin were prepared using commercial 2-chloro-5-nitropyridine (**VII**) and DIEA as the base. Darkening of the support was observed during reaction. Reaction progress was followed by the Kaiser test for amino group. It was shown that the reaction occurs at 90–95°C for 5–6 h, on average.



Synthesis of N-(5-nitropyridin-2-yl)amino acid amides. A solution (4 ml) of 0.39 (3 mmol) of DIEA in absolute DMF was added to 0.5 g of the Rink resin containing an unprotected amino acid. After 10 min, 0.73 g (3 mmol) of 2-chloro-5-nitropyridine was added to the resin. The vial was tightly closed and heated at 95°C for 12 h with intermittent shaking. The reacted resin was successively washed on a filter with DMF (4×10 ml), DCM (2×15 ml), and MeOH (2×15 ml) and dried in a vacuum oven. The dry resin was placed in a 30-ml vial, poured with 10 ml of 50% TFA/DCM, and agitated on an orbital shaker for 1 h. The resin was separated on a glass filter and washed with absolute DCM (10 ml) and absolute MeOH (5 ml). The filtrate was evaporated on a rotary evaporator. The oily residue was poured with 5 ml of absolute MeOH and evaporated again. The procedure was repeated until TFA was removed completely. The product was purified, if necessary, by recrystallization from hexane–ethyl acetate. The yield was calculated on the basis of the average resin capacity. Thus we prepared amides **XIIIa**–**XIIIg (**30–160 mg).

Esters **XIV** were prepared in the same way on the Merrifield resin (see cheme bellow).

Synthesis of methyl esters of N-(5-nitropyridin-2-ya)amino acids. A solution of (4 ml) of 0.39 (3 mmol) of DIEA in absolute DMF was added to 0.5 g of the Rink resin containing an unprotected amino acid. After 10 min, 0.73 g (3 mmol) of 2-chloro-5-nitropyridine was added to the resin. The vial was tightly closed and heated at 95°C for 10–12 h with intermittent shaking.



XIVa–XIVe

The reacted resin was successively washed on a filter with DMF (4×10 ml), DCM (2×15 ml), and MeOH (2×15 ml), dried in a vacuum oven, and weighed. The dry resin was place in a standard 30-ml vial and poured with absolute THF (3 ml) and 2M NaOMe in MeOH (1 ml). The vial was tightly closed and shaken at room temperature for 5–7 h. The resin was separated on a glass filter, washed with 30% MeOH in THF, the filtrate was neutralized with 10% HCl, and evaporated on a rotary evaporator. The residue was treated with methanol to extract the product, and the extract was evaporated on a rotary evaporator. Thus we obtained esters **XIVa**–**XIVe (**65–100 mg).

Compounds **XIIIa**–**XIIIg** and **XIVa**–**XIVe** were characterized by the ^{1}H NMR spectra (Table 6).

CONCLUSIONS

The 4-stage solid-phase reaction procedure for the preparation of non-natural amino acids, optimized by us in a student's laboratory course, is quite convenient from the methodical viewpoint. No special equipment is necessary, the yields of the target products are high, and the isolated quantities are sufficient for full-scale characterization.

The reactions can be accomplished concurrently with variable number of amino acids and hetarylating agents (for example, in the 2×2, 2×3, or 3×3 formats). Amino acids themselves (unlike amino acids immobilized on resins) are commercially available and inexpensive. In this connection, the total number of stages in a training task should be determined by the possibilities of the training laboratory. We set ourselves the task to work out the *total cycle* (including protection and immobilization stages).

REFERENCES

1. Ermolat'ev, D.S. and Babaev, E.V., *Molecules,* 2003, vol. 8, pp. 467–471; *http://www.mdpi.org/molecules/papers/80600467.pdf.*
2. Ermolat'ev, D.S. and Babaev, E.V., *ARKIVOC*, 2005, pp. 172–178; *http://www.arkivoc.com/ark/journal/2005/I04_Zefirov/1364/1364.pdf.*
3. Larsen, S.D., Connell, M.A., Cudahy, M.M., et al., *J. Med. Chem*., 2001, vol. 44, pp. 1217–1230.
4. Vailancourt, V.A., Larsen, S.D., Tanis, S.P., et al., *Ibid*., 2001, vol. 44, pp. 1231–1248.
5. Nicolau, K.C., Hanko, R., and Hartwig, W., *Handbook of Combinatorial Chemistry*, Weinheim: Wiley–VCH, 2002, vol. 1.
6. Berger, G.H., *Synth. Commun*., 1993, vol. 23, pp. 1443–1145.
7. Carpino, L.A. and Grace, Y.H., *J. Org. Chem*., 1972, vol. 37, pp. 3404–3409.
8. Kaiser, E., Colescott, R.L., Bossinger, C.D., and Cook, P.I., *Anal. Biochem*., 1970, vol. 34, pp. 595–611.
9. Brown, D.J. and Waring, P.J., *J. Chem. Soc. Perkin. Trans*., 1974, vol. 2, pp. 204–208.
10. Brown, D.J. and Ford P.W., *J. Chem. Soc. C,* 1967, pp. 568–572.
11. James, I.W., *Tetrahedron*, 1999, vol. 55, pp. 4855–4946.
12. Kobayashi, S., Akiyama, R., and Kitagawa, H.J., *Comb. Chem*., 2000, vol. 2, pp. 4238–440.
13. Tarbell, D.S., Yamomoto, Y., and Pope, B.M., *Proc. Natl. Acad. Sci. USA*, 1972, vol. 69, pp. 780–786.
14. Greene, T.W. and Peter, G.M., *Protective Groups in Organic Synthesis*, Weinheim: Wiley–VCH, 1999, ch. 6.
15. Adlington, R.M., Baldwin, J.E., Catterick, D., and Pritchard, G.J., *J. Chem. Soc. Perkin Trans. 1*, 1999, pp. 855–866.
16. Boarland, M.P.V. and McOmie, J. F. W., *J. Chem. Soc*., 1952, pp. 3716–3722.
17. Deshpande, R.N. and Nargund, K.S., *J. Pract. Chem*., 1974, vol. 316, no. 2, pp. 349–352.
18. Chichibabin, A.E., *Zh. Ross. Fiz.-Khim. O–va*, 1914, vol. 46 (2), pp. 1236–1296.
19. Ilvespaa, A.O., *Helv. Chim. Acta,* 1968, vol. 51, no. 7, pp. 1723–1733.