NEW GENERATIONS OF OPTICALLY ACTIVE NON-PROTEINOGENIC 
α-AMINO ACIDS, SYNTHESIS AND STUDY

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The latest discoveries in medicinal chemistry increase day by day with the use of unnatural α-amino acids. As irreversible enzyme inhibitors non-proteinogenic α-amino acids are widely used in the synthesis of drugs and other bioactive molecules, since the cleaving effect of proteases and other enzymes is notably weakened in the case of substrates of unnatural origin. Thus, inclusion of non-proteinogenic amino acids into the drugs structure results in essential prolongation of drugs effect \cite{1}. Therefore, unnatural α-amino acids are used in the synthesis of drugs of different action as important pharmacologically active aglycons. Thus, a strong antibiotic \textit{Leucinostatin A}, having antitumor activity is comprised of three moieties of (S)-α-methylaminopropionic acid \cite{2}; O-methyl-L-threonine is used for the synthesis of an important physiologically active peptide 3-O-methylthreonine-oxytocin \cite{3}; β-N-amino substituted derivatives of amino acid are part of \textit{Tubercatinomycin} \cite{4}, \textit{Bleomycin} \cite{5}, \textit{Edeine} \cite{6}, \textit{Capreomycin} \cite{7}, \textit{A-19003} \cite{8} antibiotics, etc. β-Hydroxy-α-amino acids of different structures are important components of physiologically active cyclic peptides (\textit{Vancomycine}), and enzyme inhibitors \cite{9}.
Thus, for example, *D*-allo-threonine is included into the composition of *Katanosins* [10] and *Accurninatur* [11] antibiotics; (+)*-Lactacystine* [12], and *Cyclosporin* [13] contain β-hydroxyisoleucine moiety. (*S*)-Substituted cysteine is used for the synthesis of physiologically active cysteine-containing peptides [14]. Inclusion of *D*-allo-isoleucine into the antibiotic *Dactinomycin D* imparts to the drug anticarcinogenic activity [15]. Sympathomimetic drug N-carboxyphenylprolyllysine is part of the antihypertensive drug *Lysinopril* [16]; derivatives of L-lysine, L-oxypoline and *D*-phenylalanine are parts of anticancer drugs *Leuprolide* [17], *Octreotide* [18], *Tuftsin* [19]; (*S*)-2-methyl-3,4,5-trihydroxy-phenylalanine possesses antitumor activity [20], (2S,4S,4S)-4-fluoroglutamic acid is an important component of antitumor drug *Methotrexat* [21], etc. (*S*)-substituted cysteines are used for the synthesis of cysteine-containing physiologically active peptides [22]. Non-proteinogenic aliphatic *D*-amino acids are applied as intermediates for the synthesis of many chiral drugs, for example, of antidiabetic drugs Alogliptin, Linagliptin, Sitagliptin, Saxagliptin and others [23].

Non-proteinogenic amino acids have also found their application in modern organic chemistry. They can be employed as chiral material for the synthesis of natural compounds and their biologically active analogs, as reagents or ligand-catalysts. In particular, synthetic non-proteinogenic amino acids are of great importance in peptide investigations, they are included into peptide structure to limit conformational flexibility of molecules. This results in enhancement of the stability to enzymes, improves pharmacodynamics and bioavailability [24]. The role of non-proteinogenic amino acids in protein engineering is very high. They are introduced into the structure of proteins to study the structure dependence of functional peculiarity of proteins [25].

It should be noted that chiral unnatural amino acid is eligible for the use in the afore-mentioned spheres only as one enantiomerically enriched isomer. Though the microbiological methods for the synthesis and enzymatic cleavage of racemates are effective for the production of natural chiral α-amino acids, they proved to be unsuccessful in the synthesis of non-proteogenic α-amino acids. Hence, the asymmetric methods for production of chiral biologically active molecules, in particular, of amino acids of non-proteinogenic origin are urgent and demanded today.

There are four main strategies for the production of chiral α-amino acids [26]. The first strategy is selective addition of a carboxylic acid equivalent to the prochiral α-carbon of an imine, as in the asymmetric Strecker reaction (Scheme 1).

### Scheme 1

\[
\begin{align*}
\text{R} & \quad \text{HCN} \\
\text{CN} & \quad \text{CO}_2\text{H}
\end{align*}
\]
The next one is asymmetric addition of hydrogen to $\alpha$-carbon of di-dehydroamino acids as in the case of Knowles Monsanto synthesis (Scheme 2).

**Scheme 2**

![Scheme 2 Diagram](image)

The third is Corey-Link reaction, asymmetric addition of hydride to ketone: precursor of amino acid. A large number of glycine derivatives are used as precursors of electrophiles and nucleophiles followed by asymmetric addition of R-group to $\alpha$-carbon of the glycine moiety [27]. (Scheme 3).

**Scheme 3**

![Scheme 3 Diagram](image)

There are also many other approaches for the synthesis of chiral $\alpha$-amino acids, e.g. electrophilic amination of enolates, nucleophilic amination of $\alpha$-substituted amino acids, enzymatic synthesis including enzymatic cleavage of racemates, etc. [28].

Despite a large number of efficient catalytic methods for the asymmetric synthesis of amino acids, the practical application of the existing methods is limited due to a number of important factors: the complexity of synthesis and expensive catalysts, use of toxic initial products (e.g. HCN, acetone cyanohydrin or trimethylsilylcyanide) in stoichiometric ratios limiting their applied usage in large-scale productions [29]. Besides, direct hydrolysis of optically active $\alpha$-aminonitriles can lead to impairment of the optical purity of target products [30].
Thus, elaboration of more practical and applicable for the technology asymmetric methods for the synthesis of non-proteinogenic amino acids is still a topical and demanded task.

The direction of the asymmetric synthesis is successfully developing in research teams of the world-known scientists, such as M. Makoza [31], E. Carreira [32], K. Maruoka [33], M. Shibasaki [34], D. Seebach [35], U. Schollkopf [36], Yu. Belokon [37] and others who conduct the asymmetric synthesis of amino acids, amino alcohols, low molecular weight peptides and other chiral biologically active molecules using various chiral catalysts and auxiliaries.

In the present article we communicate the stoichiometric asymmetric synthesis of non-proteinogenic α-amino acids with substituents of various origin in the side-chain based on the use of Ni$^{II}$ complexes of the Schiff base of amino acids with chiral auxiliary (S)-2-N-((N`-benzyl-prolyl)aminobenzophenone (BPB).

Since 1985 the biomimetic direction of the asymmetric synthesis has been successfully developing in the SPC “Armbiotechnology” NAS RA and in the Department of Pharmacy of Yerevan State University.

Various chiral complexes of transition metal ions containing Schiff bases of amino acids and carbonyl compounds, studied in biomimetic transformation reactions of amino acids, were synthesized [1].

![Chiral complexes of transition metals.](image)

The best results in both stereoselectivity and technological parameters were recorded in case of using square-planar Ni$^{II}$ ion complexes with the Schiff base of amino acids (dehydroamino acids) and chiral auxiliary BPB (D) (complexes of Yu. Belokon) [38,39].

The first chiral auxiliaries – carbonyl derivatives of N-benzyl (S)-proline with 2-amino-benzaldehyde (BPBA), 2-aminoacetophenone (BPA) and 2-
aminobenzophenone (BPB) were obtained on the basis of the natural cyclic amino acid (S)-proline and studied in the asymmetric reactions of amino acids synthesis. The research has shown that in a series of Ni^{II} complexes of the Schiff bases of amino acids and these carbonyl derivatives, complexes based on BPB chiral auxiliary have the highest enantioselectivity. Moreover, it was shown that in the absence of benzyl substitution in the pyrrolidone fragment irrespective of the size of the aldimeine substituent (at the -C=N- bond) the enantioselectivity was equal to zero in conversion reactions of the amino acid moiety. However, in the case of N-benzylproline-containing chiral auxiliaries the stereoselectivity in synthesis of amino acids increases with the increase of substituents sizes at the aldimeine carbon atom of complexes: ee is ~20% in case of 2-aminobenzaldehyde (BPBA), ~50% in case of 2-aminoacetophenone (BPA) and ~90% in case of 2-aminobenzophenone (BPB) ligands [1,40].

Complexes have a number of technological advantages:

- have the highest Cα-H acidity of amino acid moieties and electrophilicity of the C=C bond of dehydroamino acid moieties providing quantitative procedure of the C-alkylation reaction;
- well soluble in organic solvents and practically insoluble in water solutions, thus facilitating the stages of isolation of the target and intermediate complexes from the reaction medium;
- easily destroyed in a medium of weak acids (~0.5N HCl) significantly simplifying isolation of the target amino acids from alkylated complexes;
- the main advantage of these complexes is high thermodynamic enantioselectivity.

The difference between the energies of (S,S)- and (S,R)-diastereomers of these complexes is more than kcal/mol that is sufficient to reach high stereoselectivity. Thermodynamically less stable (S,R)-diastereomer gradually converts to a more stable (S,S)-diastereomer and in about 1 hour after start of the reaction, the excess of (S,S)-diastereomer exceeds 95% (Figure 2).

![Fig 2.Thermodynamic equilibrium between diastereomers.](image)

Using these complexes the efficient asymmetric synthesis of (S)-α-amino acids with various substituents in the side-chain radical was carried out. As initial amino acid synthons, Ni^{II} complexes of the Schiff base of amino acids (glycine and
alanine) and dehydroamino acids (dehydroalanine and dehydroaminobutyric acid) with chiral (S)-BPB auxiliary were used. Amino acid complexes were employed in the reactions of electrophilic C-alkylation of the amino acid moiety with formation of α-substituted α-amino acids and dehydroamino acid complexes – in the reactions of nucleophilic Michael addition with formation of β-substituted α-amino acids (see Scheme 4).

![Scheme 4](image)

Various enantiomerically enriched α- and β-substituted (S)-α-amino acids containing aliphatic and aromatic substituents of different structures have been synthesized by this Scheme. A total of about 80 new non-proteinogenic α-amino acids not described in the literature have been synthesized.

According to the average data, the stereoselectivity of synthesis of α- and β-substituted α-amino acids makes up 90%.

Chiral NiⅡ complexes of the Schiff bases of dehydroamino acids with BPB, having an active electrophilic C=C bond, prove to be suitable synthons to include heterocyclic groups into the side chain of amino acids. The asymmetric synthesis of a wide range of enantiomerically enriched heterocyclic substituted non-proteinogenic α-amino acids of (R)- and (S)-absolute configuration was carried out through nucleophilic Michael addition of various heterocyclic amines and thiols to the dehydroamino acid moiety of these complexes followed by decomposition of diastereomeric mixtures of complexes of addition products and isolation of the target amino acids (Scheme 5).
It should be noted that heterocyclic α-amino acids are regarded as interesting objects for pharmaceutical research since they are alien for the body in both structure and nature of heteroatoms [41].

Under this universal Scheme we have succeeded to synthesize more than 50 new enantiomerically enriched non-proteinogenic amino acids containing various heterocyclic substituents in the side-chain radical. Furthermore, the developed strategy enables to include heterocyclic radicals of very different structures and nature into the structure of amino acids [42].

Ni\textsuperscript{II} complexes of the Schiff base of amino acids and BPB are suitable precursors for setting up a small-scale production of optically active non-proteinogenic amino acids since 90% stereoselectivity and 1-2 hour duration for the asymmetric reactions are good technological parameters [43].

However, these factors are insufficient to use these complexes in producing isotope-labeled amino acids that are employed in PET diagnostics as radiotracers. The reason for this is the short half-life of isotopes. Thus, the time of half-life of isotopes \textsuperscript{18}F and \textsuperscript{11}C most frequently used in the composition of PET-radiopharmaceutical preparations is 109 and 29 min, respectively, while the time of asymmetric reactions for amino acids syntheses with use of Ni(II) complexes with chiral auxiliary BPB is about 1-2 hours.
Hence, to obtain isotope-labeled amino acids, it is necessary to develop transient and highly selective techniques for the asymmetric synthesis of amino acids. To solve this problem, we modified complexes of Prof. Belokon by including additional substituents into the phenyl groups of N-benzylproline and aminobenzophenone moieties. For this, different modified Ni(II) complexes of Schiff base of amino acids containing electron-donating and electron-withdrawing substituents in these phenyl groups were synthesized (Figure 3) [1].

![Fig. 3 Structure of modified NiII complexes.](image)

All these complexes were tested in the reactions of asymmetric synthesis of amino acids in both the reactions of electrophilic C-alkylation of amino acid complexes and in the reactions of nucleophilic addition of dehydroamino acid complexes. The best results in both stereoselectivity and duration of asymmetric reactions were obtained in case of using modified NiII complexes of Schiff base of amino acids and dehydroamino acids containing chlorine or fluorine atom in position 2 of the phenyl group of N-benzylproline moiety; the enantiomeric excess of the main diastereomers of alkylated complexes made up 97% on average and the time of asymmetric reactions – 3-30 min [44].

Using these modified complexes, transient methods for the asymmetric synthesis of α-amino acids into which various aliphatic and aromatic substituents are easily included have been later developed. This strategy is being successfully used in the Institute of Human Brain of the Russian Academy of Sciences when synthesizing 18F-labeled amino acids, particularly, (2-18F-fluoro-L-tyrosine (2-18F-FTYR), 3,18F-fluoro-L-α-methyl-tyrosine (3-18F-FAMT), O-2-[18F]fluoroethyl-L-tyrosine (18F-FET), 3,4-dihydroxy-6-[18F]fluoro-L-phenylalanine (6-[18F]-L-DOPA) [45] (Figure 4).
In recent years unsaturated α-amino acids are of special interest in the pharmaceutical industry. Amino acids with unsaturated bonds in the side-chain radical are interesting objects for the pharmaceutical research as they are active inhibitors of metalloproteases, Endophelium-converting enzymes and other enzymes. Specifically, acetylenic amino acids are in the spotlight of such well-known Companies as Procter and Gamble Pharmaceuticals, Novartis Pharmaceuticals and others.

It should be mentioned that the number of known unsaturated amino acids is limited. There are few natural acetylenic amino acids, isolated mainly from fungi, which have a capacity to inhibit enzymes. As to the synthetic analogs of unsaturated amino acids – the literature describes only a few of such amino acids, moreover in the form of inactive racemates [46].

In this connection, we set the task to use the unique capacities of chiral Ni(II) complexes of Schiff bases of amino acids and BPB to include unsaturated bonds into the side group of the amino acid moiety. For this, the appropriate propargylglycine and propargylalanine complexes were synthesized by C-alkylation of the amino acid moiety from Ni(II) complexes of the Schiff base of glycine and BPB (Scheme 7).
Propargylglycine and propargylalanine complexes were investigated as the starting amino acid synthon to study other unsaturated α-amino acids. Using coupling Sonogashira, Heck and Glaser reactions, different unsaturated amino acids, containing acetylene, allyl and other unsaturated groups in the side chain, were synthesized from propargylglycine and propargylalanine complexes. Dimeric amino acids containing acetylene groups as coupling links were also synthesized [47]. We also succeeded in synthesis of heterocyclic substituted amino acids containing acetylene bonds as a linking bridge (Scheme 8).

Thus, using the unique properties and enantio capacities of square-planar Ni\textsuperscript{II} complexes of Schiff base of amino acids and chiral carbonyl derivative of (S)-proline ((S)-BPB) more than 150 new enantiomerically pure non-proteinogenic (S)-
α-amino acids containing alkyl, propyl and unsaturated groups of very different structure in the side-chain radical have been synthesized.

Use of such amino acid complexes based on chiral derivative of (R)-proline [(R)-BPB] will enable to conduct the asymmetric synthesis of similar non-proteinogenic α-amino acids of (R)-absolute configuration.

Chemical structures, absolute configurations, the degree of chemical and enantiomeric purity of the synthesized non-proteinogenic amino acids and their intermediate complexes were investigated and established by modern physicochemical methods of analysis (1H-NMR, 13C-NMR, X-ray structural analysis, elemental analysis, chiral HPLC and GLC, IR, polarimetric measurements, etc.).

Specific data of synthesized compounds are not cited in this paper due to a large volume, however all analyses data unequivocally confirm chemical structure and absolute configuration, as well as high chemical and optical purity (ee>99%) of the synthesized amino acids.

Based on the obtained data an efficient technology for production of optically active non-proteinogenic α-amino acids has been developed. It has the following technological advantages:

- The technology is universal and makes it possible to obtain different non-proteinogenic amino acids on one production line using the same starting complex.
- High stereoselectivity. De of the main diastereomer of the alkylation product practically for all reactions is 90% and higher. This allows to produce optically pure amino acid with more than 98-99% enantiomeric purity by one crystallization.
- Regeneration of chiral auxiliary. After each synthesis, the initial chiral auxiliary BPB regenerates with a quantitative chemical yield and complete retention of the starting optical activity. This allows to use it many times in the reactions of asymmetric synthesis of amino acids.
- **Profitability of the technology.** The technology enables to obtain expensive and important non-proteinogenic amino acids from available and cheap raw material - glycine and D,L-alanine that cost about 10 US dollars per 1 kg of the substance. And the average cost of the products - synthesized non-proteinogenic amino acids is 250 US dollars per 1 gram of the sample. By this price we realize our amino acids in the European market.

Using the developed methods a small-scale production of optically active non-proteinogenic α-amino acids has been set up in the Scientific and Production Center “Armbiotechnology”.

The technological line on the example of production of α-amino acids with unsaturated groups in the side-chain radical is presented in Figure 5.
I. Synthesis of Ni\textsuperscript{II}-(S)-BPB-Gly complex (1); II. Filtration of complex 1; III. Synthesis of Ni\textsuperscript{II}-(S)-BPB-(S)-PGly (2); IV. Filtration of complex 2; V. Synthesis of Ni\textsuperscript{II}-(S)-BPB-(S)-PGly (3) or Ni\textsuperscript{II}-(S)-BPB-(S)-PGly-R’ (4); VI. Decomposition of 3 or 4 complex; VII. Filtration of (S)-BPB; VIII. Regeneration of initial (S)-BPB; IX. Demineralization of the target amino acid; X. Evaporation of amino acid solution and crystallization.

Fig. 5. Technological scheme for preparative production of optically active \(\alpha\)-amino acids.

On this technological line various enantiomerically pure non-proteinogenic \(\alpha\)-amino acids realized in the European market – ACROS ORGANICS (Belgium), IRIS BIOTECH (Germany), etc. are regularly produced.

At the same time medico-biological studies of the synthesized new amino acids were conducted.

Screening of the synthesized amino acids and peptides based thereon identified compounds with a whole set of biological properties. Among compounds having antibacterial activity the following amino acids: \((S)\)-\(\beta\)-[4-allyl-3-(furan-2-yl)]-5-thioxo-1,2,4-triazol-1-yl]-\(\alpha\)-alanine, \((S)\)-\(\beta\)-[4-allyl-3-(2’-chlorophenyl)]-5-thioxo-1,2,4-triazol-1-yl]-\(\alpha\)-alanine and \((S)\)-\(\beta\)-[4-allyl-3-(pyridin-3’-yl)]-5-thioxo-1,2,4-triazol-1-yl]-\(\alpha\)-alanine, as well as the following dipeptides: N-formyl-methionyl-alanyl-(S)-\(\beta\)-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-\(\alpha\)-alanine and N-formyl-(S)-methionyl-(S)-\(\beta\)-[4-phenyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-\(\alpha\)-alanine can be mentioned. It is necessary to note that these compounds also inhibit growth of antibiotic-resistant strains of \textit{E.coli} [48].

In the study of mutagenic/antimutagenic properties of compounds it is shown that tripeptides N-formyl-(S)-methionyl-glycyl-(S)-\(\beta\)-[4-phenyl-3-propyl-5-thioxo-
1,2,4-triazol-1-yl]-α-alanine and (S)-β-[4-allyl-3-(pyridin-4'-yl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine and amino acid (S)-β-[4-allyl-3-(pyridin-4'-yl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine several times increase the frequency of spontaneous and N-methyl-N′-nitro-N-nitrozoguanidine-induced mutations [49]. A number of compounds, such as (S)-β-[4-allyl-3-butyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine, (S)-β-[4-allyl-3-((3′)-hydroxypropyl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine, (S)-β-[4-propyl-3-isobutyl-5-thioxo-1,2,4-triazol-1-yl]-α-alanine and (S)-methoxy-5-nitrophenyl-alanine have the ability to reduce the frequency of NG-induced mutations, i.e. have antimutagenic properties [50].

Among new synthetic amino acids and peptides inhibitors of some enzymes including inhibitors of serine proteases and metalloproteases were revealed. Out of investigated compounds the strongest inhibitors of proteinase K proved to be R-, S-stereoisomers of allylglycine (IC50= 5.36 mM, IC50= 6.02 mM), (2S,3R)-β-hydroxyxyleucine (IC50= 3.21 mM) and (2R,3S)-β-hydroxyxyleucine (IC50= 3.43 mM), allo-O-ethylthreonine (IC50= 3.86 mM), (R)-α-methyl-β-phenylalanine (IC50= 3.02 mM).

The strongest inhibiting effect on tripsin exhibited (2R,3S)-β-hydroxyxyleucine (IC50=1.9 mM) and (2S,3R)-β-hydroxyxyleucine (IC50=1.1 mM), as well as dipeptides N-formylmethionyl-(2S,3R)-β-hydroxyxyleucine (IC50=0.1 mM), N-formylmethionyl-(2R,3S)-hydroxyxyleucine (IC50=0.2 mM) and N-formylmethionyl-(S)-allylglicine (IC50=3 mM) [50]. Furthermore, both stereoisomers of β-hydroxyxyleucine and N-formylmethionyl-(S)-allylglicine also have antibacterial properties.

Tripeptide alanylglycyl-(S)-β-[4-allyl-3-(pyridin-3′-yl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine is collagenase inhibitor (IC50= 0.11 mM). Kinetic studies showed that alanylglycyl-(S)-β-[4-allyl-3-pyridin-3′-yl]-5-thioxo-1,2,4-triazol-1-yl] was a competing inhibitor [51]. (S)-β-(N-enzylamino)alanine inhibits activity of bacterial aminotransferases.

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Experimental part

1H NMR spectra were recorded on a “Mercury-300 Varian” (300 MHz). Optical rotations were measured on “Perkin Elmer-341” polarimeter. All the reagents used were purchased from “Aldrich”. Enantiomeric purity of amino acids was determined by HPLC on the chiral phase, Diaspher-110-Chirasel-E-PA 6.0 μm 4.0×250 mm.

General procedure for the synthesis of NiII complexes of Schiff bases of amino acids with chiral auxiliaries. A solution of 1 eq. BPB, 5 eq. of amino acid and 2 eq. of Ni(NO3)2x6H2O in absolute MeOH was heated to 40 °C and a solution of 4 eq. KOH in MeOH was added and the whole was stirred at 50-60°C for 2 h (in
case of obtaining glycine complex the reaction time is no more than 1 h). The reaction was monitored by TLC on SiO$_2$, in the system CHCl$_3$/(CH$_3$)$_2$CO (3:1) following the disappearance of the spot of the initial BPB. Upon completion of the reaction, the mixture was neutralized with AcOH in 45 ml of water to pH 5-6. The precipitate was filtered off and the produced complex recrystallized from methanol.

**General procedure for the asymmetric addition of nucleophiles to the double C=C bond of the complex.** 1 eq. of the complex was dissolved in CH$_3$CN and in the argon stream were added 2 eq. of K$_2$CO$_3$ and 1.2 eq. of nucleophile. The addition reaction was monitored by TLC on SiO$_2$, in the system of solvents CHCl$_3$–CH$_3$COCH$_3$ (3:1). After establishment of a thermodynamic equilibrium between diastereoisomers, the reaction mixture was filtered, the K$_2$CO$_3$ precipitate washed with chloroform and chloroform filtrate evaporated to dryness.

**General procedure for complex alkylation.** To 1 eq. of Ni$^{II}$-(S)-BPB-S-PGly complex in DMF were added 3 eq. of finely ground NaOH, 3 eq. of alkylhalogenide. The reaction was monitored by TLC [SiO$_2$, CH$_3$COOEt/CH$_3$COCH$_3$/C$_7$H$_{16}$ (1/1/1)] following the disappearance of traces of the initial complex. Upon completion of the reaction, the mixture was precipitated from water, filtered, the alkylation product crystallized from methanol.

**Decomposition of the complex and isolation of the target amino acid.** Dry precipitate of the complex was dissolved in CH$_3$OH and slowly added to a solution of 6N HCl heated to 60ºC. After disappearance of the typical for complexes red coloration, the solution was concentrated under vacuum, water added and the initial (S)-BPBxHCl filtered. From water layer the amino acid was demineralized by passing the solution through ion-exchange column with cationite Ku-2x8 in H$^+$ form, the resin was washed with 5% NH$_4$OH. Eluate was concentrated under vacuum and the amino acid crystallized from aqua-alcohol solution.

**General procedure for determining enantiomeric yield of non-proteinogenic amino acid by HPLC analysis.**

In our work we used liquid chromatograph “Waters 2695 Separations Module” (USA) with ultraviolet detector “Waters 2487”, separation column “Nautilus-E” 4.0 x 250 mm, 5 $\mu$m for enantiomers of non-proteinogenic amino acids. Separation of enantiomers of non-proteinogenic amino acids was carried out in isocratic elution mode, with 0.1 M aqua solution of NaH$_2$PO$_4\times$2H$_2$O and CH$_3$CN (80:20 rev./rev.) as a mobile phase, 0.5 ml/min flow rate, detection was carried out with 200 nm wavelength, column temperature -30ºC, injection volume – 10 $\mu$l. Chemicals and eluents of “Sigma-Aldrich” with gradient grade > 99.9% were used for HPLC. 1 mg of the tested sample was dissolved in 1 ml of methanol in special test tubes for analysis, the sample was then inserted into a special section of chromatograph designated for the tested samples and analysis was carried out according to the developed procedure. The injection volume was 10 ml for each analysis. The results of analysis were reflected on the computer screen as a chromatogram and software enabled to automatically integrate the obtained peaks.
АННОТАЦИЯ

В данной статье посвящена стехиометрическому асимметричному синтезу небелковых α-аминокислот с различной природой заместителей в боковой цепи, основанному на использовании NiІІ комплектсов оснований Шиффа α-аминокислот с хиральным вспомогательным реагентом (S)-2-N-(N’-бензилпролил)аминобензофеноном (BPB).

Начиная с 1985 г. биомиметическое направление асимметрического синтеза удается развивать в НПЦ «Армбиотехнология» НАН RA и на кафедре фармации Ереванского государственного университета. Были получены различные хиральные комплексы ионов переходных металлов с содержанием основания Шиффа α-аминокислот и карбонильных соединений, которые исследовались в асимметричных реакциях превращения аминокислот в качестве хиральных катализаторов или вспомога-
рельных реагентов. Однако наилучшие результаты как по стереоселективности, так и по технологическим параметрам были зафиксированы в случае использования плоско-квадратных комплексов иона NiІІ с основанием Шиффа аминокислоты или дегидроаминокислоты и хирального вспомогательного реагента ВРВ. Комплексы аминокислот (глицина и аланана) использовались в реакциях электрофильного С-алкилирования аминокислотного остатка с образованием α-замещенных α-аминокислот, а комплексы дегидроаминокислот (дегидроаланина и дегидроаминомасляной кислоты) — в реакциях нуклеофильного присоединения по Михаэлю с образованием β-замещенных α-аминокислот.

В результате исследований удалось синтезировать различные α- и β-замещенные α-аминокислоты с содержанием заместителей в боковом радикале; стереоселективность синтеза при этом превышает 90%.

Анализ литературных данных показывает, что в последнее время в фарминдустрии особый интерес представляют ненасыщенные α-аминокислоты, поэтому перед нами была поставлена задача использовать уникальные особенности хиральных NiІІ комплексов основания Шиффа аминокислот и ВРВ для внедрения ненасыщенных связей в боковую группу аминокислотного остатка. Для этого путем С-алкилирования глицинового остатка NiІІ комплекса его основания Шиффа с ВРВ были получены соответствующие комплексы пропаргилглицина и пропаргилаланина, которые использовались в качестве исходного аминокислотного предшественника для получения ненасыщенных α-аминокислот.

С использованием реакции Соногашира, Хека и Глейзера из комплексов пропаргилглицина и пропаргилаланина синтезировались предшественники различных ненасыщенных α-аминокислот, содержащие ацетиленовые, аллильные и другие ненасыщенные группы в боковой цепи. Были синтезированы также бис-аминокислоты, содержащие в качестве связывающего звена ацетиленовую группу.

Итого, с использованием уникальных свойств и энантиоспособности плоско-квадратных NiІІ комплексов основания Шиффа аминокислот и хирального карбонильного производного (S)-пролина [(S)-ВРВ] было синтезировано более 150 новых энантиомерно чистых небелковых (S)-α-аминокислот, содержащих алифатические, ароматические и гетероциклические группы разной природы в боковом радикале.

Следует отметить, что использование таких же аминокислотных комплексов на основе хирального производного (R)-пролина [(R)-ВРВ] позволит осуществить асимметрический синтез аналогичных небелковых α-аминокислот (R)-абсолютной конфигурации.

На основании полученных данных была разработана эффективная технология производства оптически активных небелковых α-аминокислот.

Разработанная технология внедрена на опытно-пилотной установке Научно-производственного центра «Армбиотехнология» и организовано малотоннажное производство оптически активных небелковых α-аминокислот.

Одновременно проводились медико-биологические исследования синтезированных новых соединений. Скрининг синтезированных небелковых аминокислот и пептидов на их основе выявил соединения, об-
ладающие целым рядом биологических свойств. Среди соединений, проявляющих сильную антибактериальную активность, можно выделить, например, аминокислоты (S)-β-[4-аллил-3-(фуран-2-ил)-5-тиоксо-1,2,4-триазол-1-ил]-α-аланин, (S)-β-[4-аллил-3-{2'-хлорфенил}-5-тиоксо-1,2,4-триазол-1-ил]-α-аланин и (S)-β-[4-аллил-3-(пиридин-3'-ил)-5-тиоксо-1,2,4-триазол-1-ил]-α-аланин, а также дипептиды N-формил-(S)-метионил-(S)-β-[4-аллил-3-пропил-5-тиоксо-1,2,4-триазол-1-ил]-α-аланин и N-формил-(S)-метионил-(S)-β-[4-фенил-3-пропил-5-тиоксо-1,2,4-триазол-1-ил]-α-аланин.

При исследовании мутагенных/антимутагенных свойств соединений было показано, что трипептиды N-формил-(S)-метионилятрицил-(S)-β-[4-фенил-3-пропил-5-тиоксо-1,2,4-триазол-1-ил]-α-аланин, (S)-β-[4-аллил-3-(пиридин-4'-ил)-5-тиоксо-1,2,4-триазол-1-ил]-α-аланин и аминокислота (S)-β-[4-аллил-3-{пиридин-4'-ил}-5-тиоксо-1,2,4-триазол-1-ил]-α-аланин также повышают частоту спонтанных и НГ (N-метил-N'-нитро-N-нитрозогуанидин) индуцированных мутаций в несколько раз.

Среди новых синтетических аминокислот и пептидов выявлены также ингибиторы некоторых ферментов, включая ингибиторы сериновых протеаз и металлопротеаз.

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