1
Non-Proteinogenic α-Amino Acids, Natural Origin, Biological Functions

α-Amino acids are an important class of physiologically and pharmacologically active compounds. There are more than 1000 different amino acids in microbial cells and plant tissues. However, only 26 of them are found in protein compositions, from which only 20 amino acids can be considered typical components of proteins.

In recent years, the need for significant amounts of α-amino acids has been steadily increasing due to their extensive use in biotechnology, medicine, food, microbiology, and other areas of science and technology [1, 2]. If in the past, the need for most of α-amino acids was met by obtaining them from protein *hydrolysates* or other natural sources, from the second half of the twentieth century microbiological and synthetic directions of obtaining α-amino acids have been intensively developed.

Selection of a particular method for producing amino acids is mainly determined based on the requirements to chemical and optical purity of the final products and the area of their further use.

Synthetic methods can be considered general only if starting materials necessary for the synthesis are readily available, and reaction conditions and experimental techniques at each stage of the synthesis are similar for all amino acids. However, this is not always possible because the side chains of amino acids can have diverse structures. In addition, the main drawback of the achiral methods of chemical synthesis is the formation of amino acids in the form of racemic mixtures that could be separated on their optically active antipodes by enzymatic or microbiological methods only in the case of protein α-amino acids. In connection with this, achiral chemical methods for amino acid synthesis have found a practical application only for the production of several protein α-amino acids.

Despite this, the current total production of α-amino acids worldwide is about half a million tons per year. A large-scale production of mainly protein amino acids is due to their wide use in medicine, agriculture (growth-stimulating food additives), and food industry (flavoring substances and preservatives). The practical importance of individual amino acids is proved by the scale of their biotechnological and chemical production: tryptophan is produced in the amount of 0.2 – 0.3 thousand tons, glycine at 7 – 10 thousand tons, lysine at about

Asymmetric Synthesis of Non-Proteinogenic Amino Acids, First Edition. Ashot S. Saghyan and Peter Langer.

50 thousand tons, methionine at 150–200 thousand tons, glutamic acid at more than 200 thousand tons per year, and so on.

Specifically, methionine is used in medicine for the treatment and prevention of hepatotoxicity and diabetes, while a mixture of methionine and cysteine is used for the treatment of different kinds of poisoning. A mixture of glycine and glutamic acid is used to control gastric acidity. Pure glutamic acid is used for the treatment of CNS disorders (epilepsy, psychosis in children with polio, and mental retardation), and its sodium salt as flavoring and preservative in food. Vitamin B_3 (pantothenic acid), which contains a fragment of the nonprotein amino acid β-alanine (3-aminopropionic acid) is used in polyneurites, dermatoses, bronchitis, venous ulcers. Nonprotein γ-aminobutyric acid, detected in mammalian brain in 1950, acts as a mediator in the transmission of nerve impulses. γ-Aminobutyric acid (GABA) (aminolon, gammalon) is used to treat nervous system disorders, speech disorders, memory loss, cerebral vascular atherosclerosis, and mental retardation in children. 6-Aminohexanoic acid (ε-aminocaproic acid) is used in medicine to stop severe bleeding, as it helps in effective blood clotting.

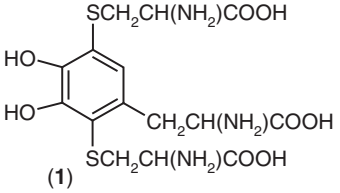
Several oligomers of α-amino acids play an important role in body functions, and some of them are used in medical practice. Thus, methyl ether of L-asparagyl-L-phenylalanine dipeptide (aspartate, aspartame) is used for diabetes as low calorie sugar substitute (150 times sweeter than glucose); a natural antibiotic *Gramicidin*, *S*-cyclic decapeptide – $[\text{Val-Orn-Leu-(D)-Phe-Pro}]_2$, produced by *Bacillus brevis*, has bacteriostatic and bactericidal action and is used to treat wounds, burns, and inflammatory diseases. It is also interesting to note that this antimicrobial peptide includes a D-form of phenylalanine. Recently, a number of small natural peptides (of leather tree frogs, snails ganglion, and poison spiders), containing one or two D-amino acids were isolated. It has been found that the D-form of the amino acid moiety in such peptides greatly increases their resistance to hydrolytic action of exo- and endoproteases. This fact is taken into account when oligopeptide drug substances with prolonged action are created [3].

Organisms can vary greatly in their metabolism because of the differences in their amino acid structure. Lately, researchers are more and more attracted by nonprotein α-amino acids with unusual structures. These include those amino acids that do not exist in the main chains of the proteins due either to the lack of specific tRNA or corresponding triplet codon or to the fact that nonprotein amino acids are not subject to a posttranslational modification. Many of these compounds are the end products of secondary metabolism, others occur as intermediates or as a result of metabolism or detoxification of foreign compounds. Due to the nature of bacterial metabolism, formation of many new compounds is possible by biosynthetic processes by adding the corresponding cell compounds to the substances of nutrient medium. These unusual amino acids can be also obtained synthetically; however, the number of "artificially" obtained amino acids of unusual structure is limited in the literature.

In essence, the nonprotein amino acids are functionally substituted derivatives of protein amino acids (substituted by α-NH_2, α-COOH, SH, OH, β, and γ-COOH,

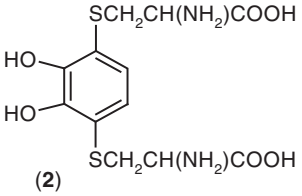
δ-NH_2, imidazole, guanidine groups, etc.) and C-alkylated analogs (α, β, γ, etc.) with a variety of aliphatic, aromatic, and heterocyclic substituents.

One of the first isolated and identified nonprotein amino acid is *dicysteinyldopa* [4]. Study of a major constituent of yellow pigment *Tapetum*, isolated from a sea pike *Lepisosteus*, revealed a new sulfur-containing product, which was purified by chromatography (*Sephadex* L1120, *Dowex* 50) and identified by physicochemical methods of analysis. Spectral analysis showed the presence of sulfur-containing *ortho*-diphenyl amino acid. After reductive hydrolysis of the isolated substance in hydrochloric acid, cysteine and dihydroxyphenylalanine (DOPA) in a ratio of 2/1 attached by thioester bond were obtained as the main products (^{1}H NMR data) (**1**).



(**1**)

This structure (**1**) was partially confirmed by biological synthesis. Tyrosinase oxidation of L-DOPA in the presence of an excess L-*cysteine* resulted in the same amino acid with 5- and 3-*S*-*cysteinyldopa*, indicating the substitution in positions 2 and 5 of the aromatic ring. Under the same conditions, catechol and cysteine formed 3,5-cysteinylcatechol and 3,6-*S*,*S*-dicysteinylcatechine (**2**), which is an additional argument in favor of the 3,5-substituted phenyl ring (3,6-*S*,*S*-dicysteinylcatechine – symmetric structure of compound **1**).



(**2**)

(2 *S*,2^1*S*,2^{11}*S*) – absolute configuration of the isolated product was established by comparing the data of polarimetric measurements of natural and synthetic product samples obtained from L-DOPA and L-cysteine.

The unusual amino acid, 2,4-diamino-3-methylbutyric acid [5], was found in the amino acid composition of root nodules of Lotus plant, which is produced by the bacterial strain of *Rhizobium.* Chromatographic and spectral analysis (NMR, mass spectroscopy, and chiral Gas-liquid chromatography (GLC) of the fraction isolated from the acid hydrolysate of the ethanol extract of this plant by ion-exchange methods (*Amberlite IR 120*, *Dowex 50*) established its (2*R*,3*S*)-absolute configuration. In the same plant species, among the protein amino acids, ninhydrin-positive compounds with unusual R_f values were also found.

In general, more than 1000 nonprotein amino acids are found in nature, extracted from plants, microorganisms, and other sources. Complete information on nonprotein amino acids are presented in the book by Barrett [6].

The main sources of known nonprotein amino acids are fauna and microorganisms that are responsible for excreting many compounds into the environment [7]. Many microbiological products show antibiotic properties, and by analogy with the fungi products, contain unusual amino acids included in more complex structures such as depsipeptides [8]. In these structures, D- and L-amino acids of common and unusual nature are connected to each other by peptide as well as by other bonds with components such as carboxylic acids and hydroxy acids. These natural molecules are rich sources of new amino acids with unusual structure.

In higher plants, unusual amino acids are most often found in free state or in the form of low-molecular-weight complexes, such as with glutamic acid. The concentration of these compounds in plant tissues can be very high. Many of the unusual amino acids from plants and animals are components of a number of pigment structures [9, 10].

Bacteria and plants differ from animal organisms by the content and chemical nature of nonprotein amino acids which affect their metabolism. In products of metabolism of animal organisms, there are no secondary by-products such as nonprotein amino acids, alkaloids, phenols, and other substances that are characteristic of lower organisms and plants.

A significant portion of nonprotein amino acids in plants have aliphatic structure, with no more than six carbon atoms in chain length, although there are also large molecules. The diversity in their structures is achieved by limited branching, substitution of hydroxyl, carboxyl, and amino groups, as well as inclusion of unsaturated allene and alline groups into the molecule. Despite the variety of halogenated aromatic compounds in marine organisms [11] and the possibility of substitution of the phenolic ring by halide atoms, the discovery of such a small number of free halogen-containing aromatic amino acids in natural sources was unexpected. In fact, any type of halogenated amino acids is relatively rare among chlorine-containing bacterial products, even in algae and marine invertebrates that are known to produce proteins and other halogenated by-products [12].

In contrast to cyclic aromatic amino acids, heterocycles of both aromatic and nonaromatic series are part of many amino acids. It is expected that most of these are nitrogen-containing heterocycles, although many of them also contain oxygen or sulfur in the ring. A number of nitrogen-containing heterocyclic amino acids derived from tryptophan are substituted in the indole ring analogs. Other nitrogen heterocycles are closely related to pyrrolidone ring and are homologs of proline.

Although there are many heterocyclic imino acids, many developments are aimed at expanding a limited number of aliphatic imino-acids with pyruvate and the products derived from amino acids such as strombine and alanopine, which are added to the octopine as components of anaerobic metabolism in invertebrates [13, 14].

Based on the diversity of the structures, it is not surprising that the clear pattern of biosynthetic origin of nonprotein amino acid is difficult to predict. If we

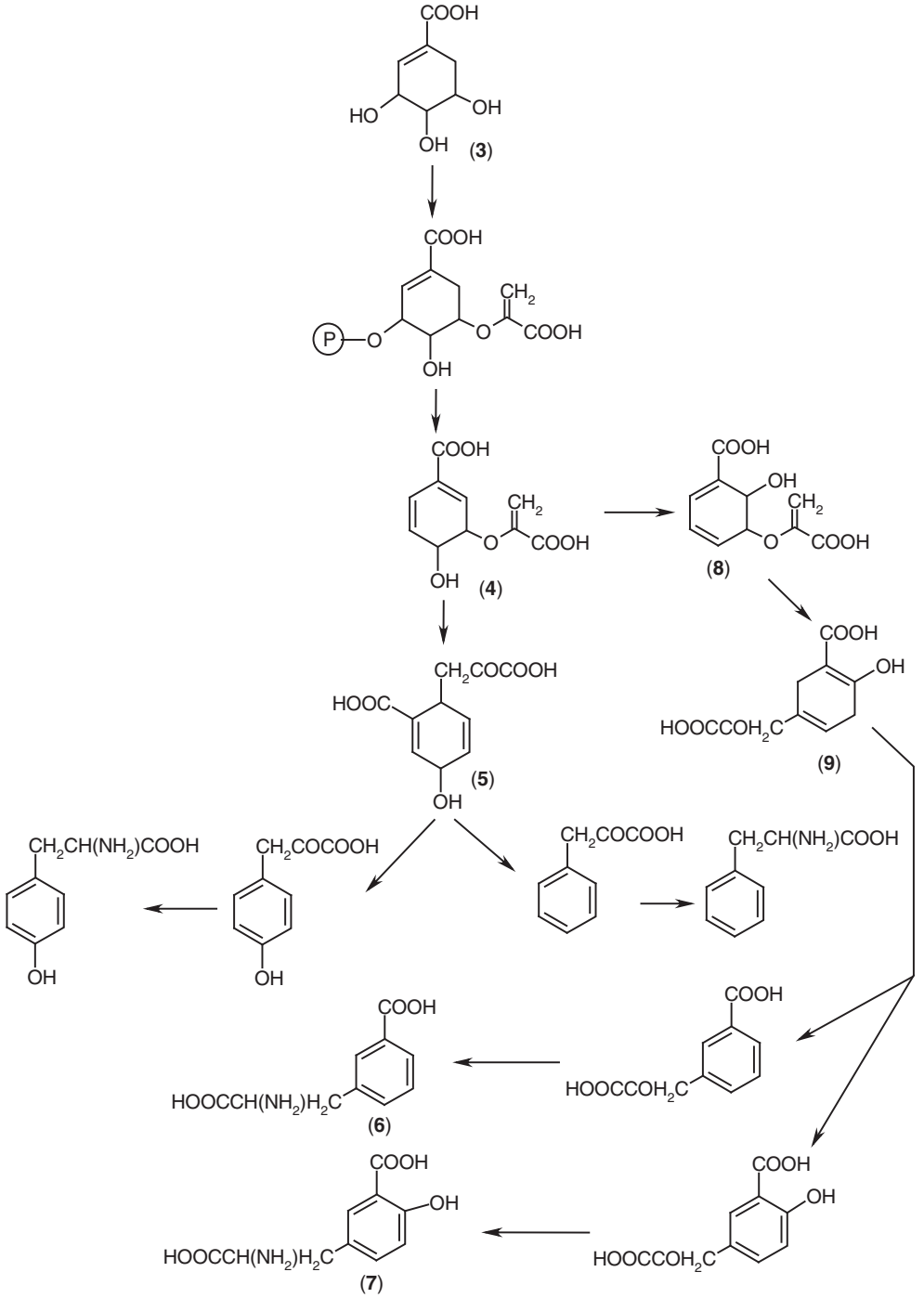
take into account the impact of environmental and other effects that lead to the accumulation of specific components, it seems likely that there are three or four general ways for the emergence of nonprotein amino acids in nature.

A possible synthesis route for many well-known products is modification of existing amino acids by mechanisms similar to those involved in posttranslational modification of protein amino acids, that is, a simple replacement of certain positions in the structure of protein amino acids. Obtaining dihydroxyphenylalanine in plants from the synthesis cycles of tyrosine [15], β-acetyl-ornithine [16], or *O*-acetylserine [17] are well-grounded examples of such modifications. The emergence of hydroxyproline [18], desmosine, and isodesmosine [19] in protein chains or pyridinoline [20] in the urine of mammals is as an example of appearance of new amino acids as posttranslational modifications of amino acids.

Thus, it is potentially possible to form many simple analogs of known amino acids by simple postsynthetic modification of 20 protein amino acids in different ways. In fact, certain amino acids are more involved in this type of modification than others. For example, in nature, there are quite many lysine analogs and very few products obtained from valine. It is obvious that the reactivity of the side chains is in favor of such modifications. The reactive thiol group of cysteine promotes the formation of a relatively large number of amino acids, and the formation of α-amino-α-carboxyl group in α-amino acids occurs by amination of keto acids in the initial stages of the synthesis of amino acids. If the formation of the functional groups in the side chain of amino acids includes several enzymatically catalyzed steps, then it is easy to imagine how the modification in intermediate stages can lead to the formation of one or more new amino acids. Perhaps, many free amino acids are formed in such a way that intermediates are actually involved in the formation of a particular amino acid. In the absence of metabolic function, the existence of these amino acids may be temporary. As examples can serve the formation of phosphoserine in the route to serine synthesis from 3-phosphorglycerate or glutamyl-γ-semialdehyde [21], or the accumulation of homoserine in the route to methionine and threonine synthesis from aspartate, or the accumulation of ornithine in the route to arginine [21]. The precursor of amino acids in the latter synthesis is glutamyl-γ-semialdehyde, while the related derivative of aspartate affords homoserine, which in turn is formed from aspartyl phosphate [21]. If we consider all possible intermediates of those types of derivatives, the list of nonprotein amino acids will be much greater.

Accumulation of homocysteine along with other unusual sulfur-containing amino acids in the blood and urine of patients with homocystinuria indicates an anomaly in the metabolism of methionine. This process can be treated as a side condensation reaction of homocysteine with serine with the formation of cystathionine, which is catalyzed by cystathionine synthase [22, 23]. Pyroglutamic acid (5-oxoproline) can appear in the urine as a result of abnormal metabolism, due to the lack of 5-oxoprolinase, which is a component of γ-glutamyl cycle [22].

The most reasoned example of abnormal metabolism is the formation of shikimate from chorismate (see Scheme 1.1). Common amino acids phenylalanine and tyrosine are synthesized from shikimate (**3**) through chorismate (**4**) and


COOH
HO
OH
OH
(3)
COOH
CH2
P
O
O
COOH
OH
COOH
CH2
O
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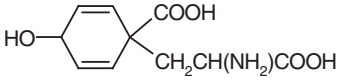
OH
(4)
COOH
OH
CH2
O
COOH
(8)
CH2COCOOH
HOOC
OH
(5)
COOH
OH
HOOCCOH2C
(9)
CH2CH(NH2)COOH
CH2COCOOH
OH
OH
CH2COCOOH
CH2CH(NH2)COOH
COOH
HOOCCH(NH2)H2C
(6)
COOH
HOOCCOH2C
COOH
OH
HOOCCH(NH2)H2C
(7)
COOH
OH
HOOCCOH2C

Scheme 1.1

prephenate (**5**). Vegetal amino acids β-(3-carboxyphenyl)alanine (**6**) and β-(3-carboxy-4-hydroxyphenyl)alanine (**7**) are synthesized by the same path directly from both chorismate and transformation of chorismate to isochorismate (**8**), and then to isoprephenate (**9**) followed by the aromatization of the ring where the modification may only exclude the decarboxylation stage [23, 24]. However, sometimes there is a deviation from this principle.

During the formation of other new aromatic amino acids from chorismate, such as the synthesis of *ρ*-aminophenylalanine in *Vigna*, amination of chorismate is observed [25]. An interesting variant of such a modification is an alternative route for the synthesis of tyrosine from chorismate in *Pseudomonas*, proceeding through a specific compound pterosine or arogenate (**10**) [26].



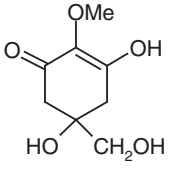
(**10**)

Although the new amino acids are the result of "tuning" of the metabolic pathways and metabolites, as described earlier, it is equally clear that this path cannot fully provide the formation of many unusual compounds.

Sometimes, even those nonprotein amino acids, structures of which seem to be very similar to the structures of protein amino acids, are products of convergent rather than parallel development. L-β-Aspartic acid in *Clostridium tetanomorphum* is formed not from aspartate, but by means of a new rearrangement of L-glutamate involving 5′-deoxyadenosylcobalamine in the aerobic enzymatic reaction [21].

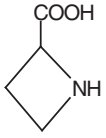
A similar methyl derivative – *erythro*-methyl-γ-glutamic acid is formed into *Gleditsia triacanthos* not from glutamic acid, but from L-leucine by the oxidation of the methyl group [27].

Currently, the actual data on the biosynthesis are available only for several groups of nonprotein amino acids. Possible ways of formation of the majority of nonprotein amino acids can only be assumed based on the known metabolic pathways for the synthesis of their protein counterparts. For example, mycosporine-like mutilins and related amino acids are probably formed by the condensation of protein amino acids of glycine, serine, and threonine with the original diketone (**11**) obtained by shikimate pathway [28]. Strombine and alanopine are formed by the condensation of pyruvate with glycine or alanine in the presence of NADH and dehydrogenase [13]. This reaction has common features with the synthesis of glutamate from α-ketoglutarate and ammonium ion by means of glutamate dehydrogenase and NADH.



(**11**)

To the unusual amino acids belongs the heterocyclic amino acid azetidine-2-carboxylic acid (**12**), which, as it was expected, is synthesized by a totally new pathway, and for its formation the hypothetical scheme of synthesis from homoserine via 2,4-diaminobutanoat to 4-amino-butanoat with subsequent cycle closure and dehydration was proposed.



(**12**)

Unusual amino acids can play many roles *in vivo*; however, presently, for most of these compounds, the specific functions are not determined. It should be noted that nonprotein amino acids of plant origin exhibit physiological activity in animals, but in some cases, these compounds cannot perform certain functions.

A significant aspect of plant metabolism is the need to retain nitrogen, thus to limit the loss of nitrogen through the synthesis of secreted nitrogen compounds. Since animals do not need to retain nitrogen, they are able to produce and excrete nitrogen-containing compounds. The nitrogen that is required for the synthesis of proteins in plants can be accumulated in large amounts in the form of alanine, asparagine, arginine, acetylornithine, allantoin, citrulline, and glutamine, which makes the protein synthesis easily traceable. However, in many cases, accumulation of high concentration of unusual amino acids does not guarantee this pathway, it is possible that some of the substances are not intended to serve as a nitrogen source or they cannot participate in metabolism.

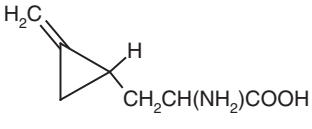
An important property of many nonprotein amino acids is their toxicity or the ability to adversely affect the metabolism of other compounds. Many plant nonprotein amino acids structurally are very similar to protein amino acids. In this sense, the accuracy of translation system is not surprising. In spite of the relative abundance of such compounds, it almost does not allow any errors, nor includes these compounds in the composition of the protein. However, nonprotein amino acid can be included in the composition of the protein, if it has a very similar structure to protein amino acids. A classic example is the introduction of azetidine-2-carboxylic acid by tissues of *Phaseolus aureus* as a replacement of proline moieties in protein [29].

Introduction of abnormal amino acid into proteins of organisms, for which this amino acid is a foreign compound, significantly alters the properties of these proteins and generally increases their toxicity.

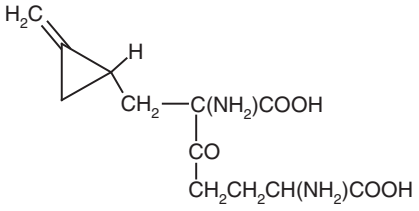
In plants, β-aminopropionitrile is formed by the decarboxylation of β-cyano alanine. It is known that β-cyano alanine and its γ-glutamyl derivative are present in the legume *Vicia sativa* and few other *Vicia* species, and are neurotoxins that cause neurological disorders [30]. Isolation of cystathionine in unusually high amounts in the urine is another manifestation of this condition, suggesting interference in the synthesis of homocysteine. Since the nature of the action of β-cyano

alanine and β-aminopropionitrile in these diseases is not fully understood, the relationship with pyridoxine and pyridoxal phosphate may be partly responsible for their toxicity. It should be noted that the lack of pyridoxine decreases the activity of amine oxidase *in vivo*, and induces the formation of defective elastin, while pyridoxal phosphate is not only required for the conversion of cystathionine to methionine but also eliminates the toxic effects of β-cyanoalanine [21, 22].

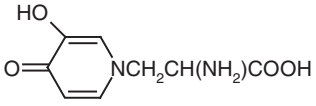
Many toxic effects on humans have been recorded in case of other plant unusual amino acids. Hypoglycines A and B (**13** and **14**) are responsible for hypoglycemia, which is caused by human consumption of unripe ackee (fruit) [31, 32]. Mimosine (**15**) causes hair and fur loss in animals and sheep fed on *Leucaena leucocephala* [33]; numerous selenium analogs of sulfur amino acids, found in many plants, also have a wide range of toxic effects on livestock.



(**13**)

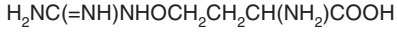


(**14**)

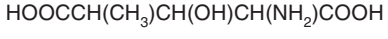


(**15**)

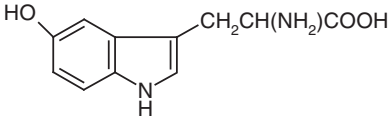
Toxic effects of nonprotein amino acids are also observed on invertebrates, especially insects. Canavanine (**16**) and β-hydroxy-γ-methylglutamate (**17**) can act as repellents (insect repellent) for certain species and can also be toxic, whereas the 5-hydroxytryptophan (**18**) and 3,4-dihydroxyphenylalanine (**19**) are toxic to beetles, weevils [34, 35], and other insects.



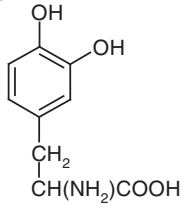
(**16**)



(**17**)



(**18**)



(**19**)

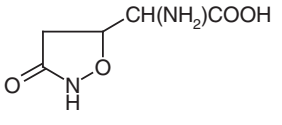
It is possible that the compound related to structure **18** and neurotransporter play a certain role because 5-hydroxytryptamine is the major neurotransmitter in the muscles of digestive tract of insects [36]. DOPA simultaneously is the predecessor of neurotransporters and cuticle of cross agents in insects; dihydroxyphenylalanine and a series of quinonoids and β-substituted ketocatechols [37, 38] are synthesized through DOPA.

High toxicity of such a wide range of plant products suggests that the cause of some nonspecific diseases in humans and domestic animals can surely arise from toxic amino acids, which are widely spread in edible plants. For example, N^8-acetyl-L-α,γ-diaminobutyric acid (**20**), which is converted to a α,γ-diaminobutyric acid toxin, inhibits urinary ornithine transcarbamylase cycle and is present in small amounts in sugar beets [39].

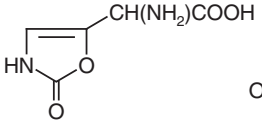
$CH_2(NHCOCH_3)CH_2CH(NH_2)COOH$

(**20**)

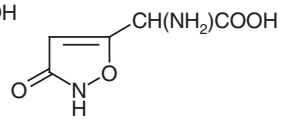
It seems that hydroxyisoleucine is responsible for the toxicity of amotoxins because its replacement by a leucine in amanulline leads to a nontoxic drug [40]. Apparently, the toxic outcome of the drug is due to its effect on RNA polymerase, inhibiting protein synthesis, which explains the slow action of the toxin. Tricholomic acid (**21**) and muscazone (**22**) are the causes of various injuries of vision, memory, and spatial or temporal orientation in humans, whereas the two compounds of *Tricholoma muscarium* and *Amanita muscaria* with ibotenic acid (**23**) from *Amanita pantherina* are potential insecticides [40]. All of these unusual amino acids comprise a fragment of osoxazole.



(**21**)



(**22**)



(**23**)

Fungi also produce nonprotein amino acids, phytotoxins, and imino acids. Lycomarasmine (**25**) and aspergillomarasmine (**26**) of *Fusarium* cause wilt in tomato leaves by forming complexes with iron ion, whereas fusaric acid (**26**) from the same source causes yellowing of leaves. Soybean leaf necrosis is caused by rizobitoksin (**27**), which blocks the conversion of cystathionine to homocysteine.

$HOOCCH_2CH(COOH)NHCH_2CH(COOH)NHCH_2CONH_2$

(**24**)

$HOOCCH_2CH(COOH)NHCH_2CH(COOH)NHCH_2CH(NH_2)COOH$

(**25**)

$HOOCCH_2CH(COOH)NHCH_2CH(COOH)NHCH_2COOH$

(**26**)

$HOCH_2CH(NH_2)CH_2OCH{=}CHCH(NH_2)COOH$

(**27**)

The action range of many antibiotics is still insufficiently elucidated, but it is obvious that many of them have a cyclic structure with a content of nonprotein

amino acids or amino acids with D-configuration. Among such compounds are gramicidins or enniatins that act on the bacterial cell membrane level by affecting the permeability of ions.

Penicillin derived from penicillamine (28) acts at the level of inhibition of peptidoglycan biosynthesis, similar to D-alanyl-D-alanine, and bind to the active site of the bacterial transacylase. Cephalosporins are related to penicillin [21] and contain D-α-aminoadipoil in the side chain. The nonprotein amino acids with antibacterial properties also include azaserine (**29**) and L-2-amino-4-(4′-amino-2′,5′-cyclohexadienyl)butyric acid [41].

$(CH_3)_2C(SH)CH(NH_2)COOH$

(**28**)

$N_2CCHCOOCH_2CH(NH_2)COOH$

(**29**)

Animals, unlike plants and microorganisms, produce little toxin-containing unusual amino acids. However, certain shellfish poisons are mixtures of peptides and proteins containing nonprotein amino acids. For example, gomarin, which is present in the venoms of some molluscs has curare-like effects [40].

Based on the foregoing, it follows that most of the biological functions of the unusual amino acids in plants and microorganisms can be associated not directly with the physiology of the organism itself, but with its relationship to other organisms in the environment. The question of physiological functions of nonprotein amino acids in higher plants is still doubtful for the majority of researchers and it is keenly debated. To a large extent, the role of such compounds is perceived as nitrogen retention.

Physiological functions of nonprotein amino acids are evaluated at various stages of plant development according to the age, time of the year, and/or stress. Studies have shown that the depletion or accumulation of specific amino acids is observed in various conditions. For example, γ-hydroxy-γ-methylglutamic acid can be detected in *Asplenium* in certain years, but not every year [42], canavanine accumulation in seeds can disappear during its growth [43], and so on. There is also no doubt that the osmotic control under stresses caused by lack of water can be achieved by changes in the concentration of free amino acids [44]. Free amino acids can also be involved in the binding of iodine – a factor that can have implications for the marine and freshwater algae [45].

Some unusual amino acids are found on the path to the synthesis of well-known plant metabolites. For example, it has been shown that the plant hormone *Ethylene* is produced from methionine through (*S*)-adenosyl methionine, and 1-aminocyclopropane-1-carboxylic acid [46].

In invertebrates, the function of some unusual amino acids is associated with energy supply to tissues under anoxic conditions. In particular, the biosynthesis of strombine, alanopine, and octopine serves to maintain the base rate of energy formation [13, 14].

The effect of different phosphorylated amino acids in invertebrates is similar to phosphocreatine effect on muscles of vertebrates. *N*-Phosphoryl arginine, phospho glycocyamine, and lombricine relate to such compounds.

On the other hand, similar to irreversible enzyme inhibitors with increased duration of action, nonprotein α-amino acids are also potentially biologically active compounds, and more recently successfully have been used in medicine, pharmacology, peptide synthesis, microbiology, and other areas of science and technology [1, 2].

According to the marketing, need for nonprotein α-amino acids annually increases by approximately 10%. In this regard, the researchers' attention is increasingly attracted to the synthesis of nonprotein α-amino acids of unusual structure with potential biological activity [47, 48].

Many nonprotein α-amino acids are part of modern high-level antitumor, hypertensive, and analgesic drugs, agents used to fight drug and alcohol addiction, and other important pharmacological agents [49, 50].

A special place among them occupy α-methyl-substituted α-amino acids, which are specific inhibitors of many enzymes capable of irreversibly binding to the active site of enzymes by covalent bonds. This principle is applied in biochemistry and enzymology to clarify the mechanism of action of many enzymes [51, 52]. α-Substituted-α-amino acids have a potent antihypertensive and antiseptic activity along with antitumor and radioprotective effects [53].

In particular, the inclusion of α-methyl-L-dihydroxyphenylalanine in the medication DOPA eliminates unwanted side effects in the treatment of Parkinson's disease [54–56]; α-methyltryptophan is used for the treatment of staphylococcal infections [57]; and α-methyltyrosine is an inhibitor for tyrosine-hydroxylase enzyme, which is responsible for tyrosine conversion to 3,4-dihydroxyphenylalanine, an important intermediate of adrenaline biosynthesis [54].

Nonprotein α-amino acids are also used as important pharmacologically active aglycones in the synthesis of various drugs. Thus, a strong antibiotic *Leucinostatin A*, having antitumor activity, comprises three moieties of (*S*)-α-methylaminopropionic acid [58]; *O*-methyl-L-threonine is used for the synthesis of an important physiologically active peptide 3-*O*-methylthreonine-oxytocin [59]; β-N-amino substituted derivatives of amino acid are part of *Tuberactinomycin* [60], *Bleomycin* [61], *Edeine* [62], *Capreomycin* [63], *A-19003* [64] antibiotics, and so on. β-Hydroxy-α-amino acids of different structures are important components of physiologically active cyclic peptides (*Vancomycine*), and enzyme inhibitors [65]. Thus, for example, D-*allo*-threonine is included into the composition of *Katanosins* [66] and *Accurninaturn* [67] antibiotics; (+)-*Lactacystine* [68] and *Cyclosporin* [69] contain β-hydroxyleucine moiety. (*S*)-Substituted cysteine is used for the synthesis of physiologically active cysteine-containing peptides [70]. Inclusion of D-*allo*-isoleucine into the antibiotic *Dactinomycin D* imparts to the drug anticarcinogenic activity [71]. Sympathomimetic drug *N*-carboxyphenylprolyllysine is part of the antihypertensive drug *Lysinoprile* [72]; derivatives of L-lysine, L-oxyproline, and D-phenylalanine are parts of anticancer drugs *Leuprolide* [73], *Octreotide* [74],

and *Tuftsin* [75]; (*S*)-2-methyl-3,4,5-trihydroxy-phenylalanine possesses antitumor activity [76]; and (2*S*,4*S*)-4-fluoroglutamic acid is an important component of antitumor drug *Methotrexat* [77], and so on.

Figure 1.1 shows the structures of some important pharmaceuticals based on nonprotein α-amino acids.

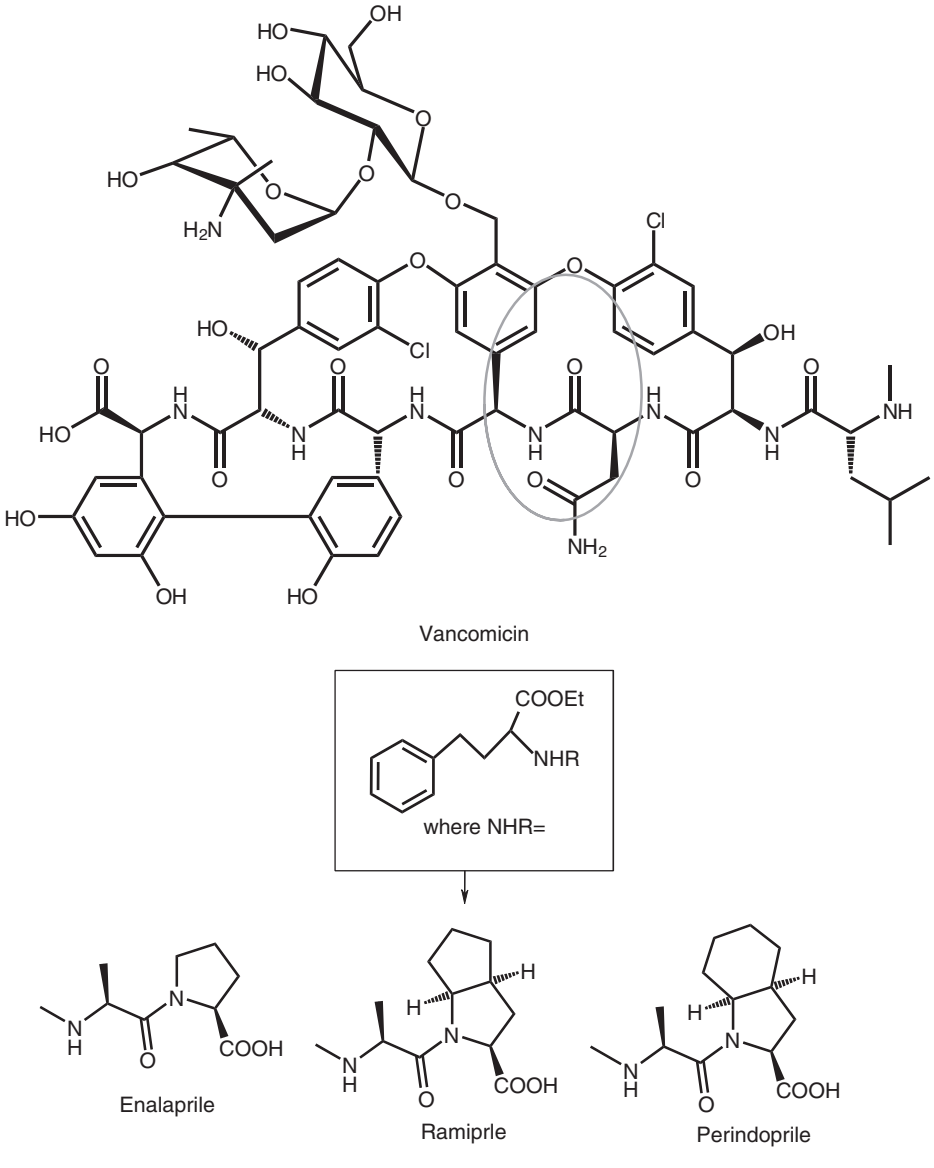


Figure 1.1 The structures of some drugs based on nonprotein amino acids.

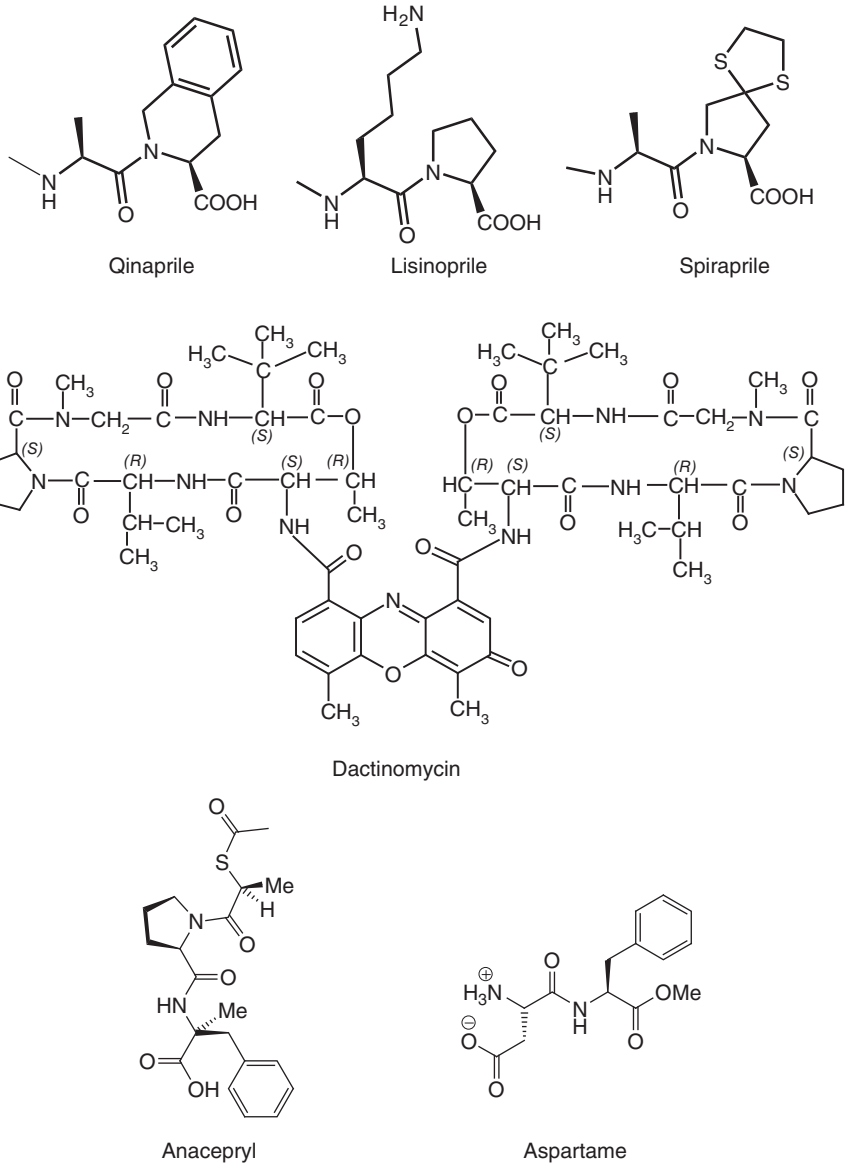


Figure 1.1 (*Continued*).

Non-natural D-amino acids widely found as common intermediates for the preparation of many chiral drugs, biologically active molecules, chiral axuliaries and some useful chiral building blocks have attracted intensive attention over the past decades regardless of the fact that natural L-amino acids are readily available. Among all the non-natural amino acids, aliphatic D-amino acids stand out as elegant intermediates for the synthesis of many chiral drugs which include

the orally administered anti-diabetic drugs in the DPP-4 inhibitor class, among which, alogliptin, linagliptin, sitagliptin, and saxagliptin are just to name a few. Moreover, due to the significance of chiral aliphatic D-amino acids, the worldwide market value of these valuable compounds is estimated at 2 billion dollars annually [78].

Nonprotein α-amino acids are also successfully used in microbiology as analogs of natural α-amino acids for the selection of their highly active strain-producers. For example, *S*-(2-aminoethyl)-L-cysteine is an effective analog of L-lysine [79], β-hydroxyleucine is an analog of L-threonine [80], *O*-methyl-L-serine is an effective analog of L-methionine [81], and β-hydroxyleucine is an analog of L-leucine [82]. Recently, in pharmaceutical and agrochemical industries, much attention is paid to methods for producing enantiomerically pure forms of biologically active compounds. Indistinguishable in their physical and chemical properties, enantiomers often sharply differ from each other in their physiological action. Thus, levorotatory nicotine in tobacco is several times more toxic than the dextrorotatory. Enantiomers of amino acids also differ in their taste. Thus, the natural amino acids L-asparagine and L-tryptophan are tasteless but L-leucine and L-tyrosine have a bitter taste, whereas their unnatural D-isomers have a sweet taste.

Enantiomers of many compounds used as drugs have various physiological effects. Thus, the racemate of antibiotic *Chloromycetin* contains 50% of physiologically inactive dextrorotatory isomer, whereas the levorotatory isomer, *Laevomycetin*, has antibiotic activity. Moreover, (+)-isopropyl noradrenaline has 800-fold more potent bronchodilator action than the (−)-isomer; (−)-5-(1,3-dimethylbutyl)-5-ethylbarbituric acid exhibits usual for barbiturates sedative action, whereas (+)-antipode causes convulsions [83]. *Thalidomide* widely known in late 1950s as a sedative drug used to eliminate insomnia and morning sickness in pregnant women has its (*S*)-(−)-antipode, which causes limb hypoplasia in newborns [84]. The drug was very popular; however, it became clear that due to its use the number of children with severe congenital deformities of the upper and lower extremities has increased. This was explained by the fact that the drug was used as a mixture of two optically active enantiomers (racemate) from which (+)-(*R*)-enantiomer has a sedative action and is nontoxic, and its (−)-(*S*)-antipode causes teratogenicity (congenital malformations) (see Figure 1.2).

According to US Senate and European Commission decisions, only enantiomerically pure drugs are needed in the drug market because the optical antipode of the

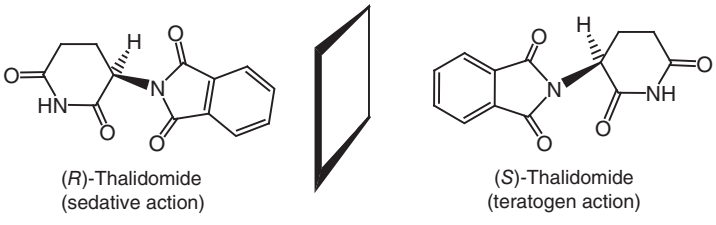


Figure 1.2 The structure of the optical antipodes of thalidomide.

pharmacologically active drugs usually has adverse effects, and at best, it remains neutral. In this regard, recently pharmaceutical companies pay more attention to the production of optically pure drugs. Thus, the total sales of enantiomerically pure drugs worldwide were more than 133 billion dollars (40% of all drugs), and the rate of production growth of chiral drugs was 18% per year [85].

Besides, the use of enantiomerically pure agrochemicals reduces their environmental impact [86]. Based on this, a large number of research laboratories have begun to develop methods for the asymmetric synthesis of chiral organic compounds used in agriculture.

A separate interesting class of nonprotein amino acids is isotopically labeled α-amino acids, which are successfully used in both biochemical research, and in positron emission tomography (PET) for fast and effective diagnosis of various diseases, including diagnosis of tumor cells in the early stages of development.

The basis of *positron emission tomography* (PET) is the use of positron-emitting radioisotope-labeled compounds. As the radioisotope undergoes positron emission decay (also known as *positive beta decay*), it emits a positron, an antiparticle of the electron with opposite charge. The emitted positron travels in the tissue for a short distance (typically less than 1 mm, but depends on the isotope), losing kinetic energy at that, until it decelerates to a point where it can interact with an electron. The encounter annihilates both electron and positron, producing a pair of annihilation (gamma) photons moving in approximately opposite directions. The most significant fraction of electron–positron annihilations results in two 511 keV gamma photons being emitted at almost 180° to each other.

Fixing these paired γ-rays with the help of PET camera's detector rings allows to determine the exact location of the annihilation place in the space.

After intravenous administration of the radiopharmaceutical drug (RPD), its distribution in the body tissues can be studied using a special external system detector (PET scanner). Therefore, for the PET method the nature and characteristics of the used isotopes are very important.

Using short-lived isotopes with high specific activity enables to conduct PET studies introducing "trace" amounts of radiopharmaceuticals without any pharmacological effect, and allows to obtain high-quality PET images with a low dose load on patients. Detecting equipment for PET is based on the registration of quantum annihilation radiation with opposing detector system (coincidence circuit).

This makes possible to determine annihilation (autocollimation) directly. Due to registering annihilation radiation using the coincidence, the conventional collimation used in single photon emission computed tomography (SPECT) is no longer necessary, which causes higher resolution of the PET method.

PET scanner comprises an array of detectors arranged in a ring in pairs included in the matching circuit. The detection system is calibrated using phantoms with a certain concentration of a radioactive isotope. Data obtained by a PET scanner are processed by "image reconstruction" special algorithms, and the result is presented in the form of distribution of the radiopharmaceutical in the body in terms of activity/volume.

Table 1.1 Main characteristics of radioisotopes and their sources.

Isotope	Half-life (min)	Nuclear reaction
^{11}C	20.4	$^{14}N(p,\alpha)^{11}C$
^{13}N	9.96	$^{16}O(p,\alpha)^{13}N$
^{15}O	2.03	$^{14}N(d,n)^{15}O$
		$^{15}N(p,n)^{15}O$
^{18}F	109.8	$^{18}O(p,n)^{18}F$
		$^{20}Ne(d,\alpha)^{18}F$

Nowadays, isotopes of the following elements ^{11}C, ^{13}N, ^{15}O, ^{18}F that make up the majority of organic molecules are widely used. These isotopes are produced in cyclotron targets by the nuclear reactions listed in Table 1.1.

Due to the very small half-lives of ^{13}N and ^{15}O, these isotopes are mainly used in the composition of the simplest compounds: [^{15}O] O_2, [^{15}O] CO, [^{15}O] CO_2, [^{15}O] H_2O, and [^{13}N] NH_3. The ^{11}C isotopes are of the greatest interest because of their longer half-lives as compared with ^{13}N and ^{15}O isotopes and in the case of ^{11}C it becomes possible to synthesize rather complex and diverse organic compounds.

The use of [^{11}C] radiopharmaceuticals in clinical practice substantially restrains the need to produce RPD in close proximity to the PET chamber for patients' examination because the half-life ($\tau_{1/2}$) is 20.4 min, and to deliver such radiotracers to the PET centers that do not have cyclotrons is impossible. Therefore, in recent PET studies, there is a trend to replace clinically used [^{11}C]RT with [^{18}F]RT. Analysis of the literature devoted to PET shows that the most promising radiotracers are labeled with ^{18}F isotope. For the preparation of ^{18}F isotope in the form of molecular fluorine, $^{20}Ne(d,\alpha)^{18}F$ nuclear reaction is used, implemented in the gas target filled with Ne in the presence of nonradioactive molecular fluorine (~0.1%) [87], and nuclear reaction of ^{18}O (p,n)^{18}F is carried out in the target filled with gaseous molecular oxygen [88].

Most of the operating cyclotron accelerators currently produce ^{18}F isotope by $^{18}O(p,n)^{18}F$ nuclear reaction. In this reaction, the ^{18}F isotope can be prepared from [^{18}O]H_2O in the form of fluoride anion, ([^{18}F]F-aq) with an activity of up to 100 GBq, with a specific activity of up to 30 000 GBq/mmol, as well as in the form of molecular fluorine ([^{18}F]F_2) in the gas target of high pressure ([^{18}O] O_2) with an activity of up to 34 GBq of and specific activity of 350–600 GBq/mmol [88].

From the sources of ^{18}F-labeled reagents, the most promising is the [^{18}F] F^-, produced by the [^{18}O] H_2O nuclear reaction, since this method has several advantages:

- higher selectivity of labeling compared with the administration of electrophilic fluorinating reagents while using;
- ability to work with ^{18}F isotope nongaseous sources;

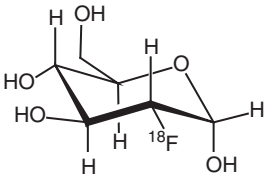


Figure 1.3 The most common radiotracer – [^{18}F] FDG.

- the possibility of obtaining radiolabel [^{18}F] F^- with high specific activity;
- easiness to purify the [^{18}F] F^- by chromatography or electrochemical methods.

Thus, [^{18}F]F^- in many studies has been selected as a fluorinating agent for the preparation of ^{18}F radiotracers. Therefore, in recent PET studies, a trend to replace clinically used [^{11}C]RT with [^{18}F]RT was observed. This is due to the fact that half-life of the ^{18}F isotope is sufficiently long as compared with ^{11}C, ^{13}N, ^{15}O isotopes, and this allows to employ the strategy of using several PET cameras served by a cyclotron located at some distance, implemented in the case of the most common radiotracer of 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) (see Figure 1.3).

On the one hand, the size of the F atom is slightly higher than the size of the hydrogen atom (van der Waals radius of hydrogen is 1.5 Å, and that of fluorine is 1.65 Å), and, on the other hand, the F atom has a significant negative inductive effect, and its introduction into the molecule significantly polarizes the part adjacent to the C–F bond.

Introduction of fluorine into the known biological compound can affect its biochemical and physicochemical properties in an unpredictable manner. In case of replacement of the hydroxyl group of the glucose molecule by ^{18}F atom, the [^{18}F]FDG is transported into tissues similar to glucose but [^{18}F]FDG metabolism in cells is stopped at the phosphorylation stage, which leads to accumulation of [^{18}F]FDG in the areas of increased energetic metabolism of glucose.

Therefore, the distribution of [^{18}F] FDG in the body tissues is described in terms of a simple pharmacokinetic model, and PET findings can be quite easily interpreted.

In accordance with the modern concepts, the use of ^{18}F-labeled amino acids (^{18}F-AAs) as diagnostic agents is determined by their accumulation in the tumor, which reflects the increased amino acid transport rate into tumor cells compared with the intact tissue. Besides, the accumulation of amino acids in the gray matter of the brain is low that advantageously distinguishes them from [^{18}F]FDG, which often gives false-positive results in cases of brain tumors [89].

Moreover, in recent studies it has been shown that amino acid transport in infected cells (foci of infection) is significantly lower than in the tumor tissue. Thus, in the inflammation site, induced in the rat's body by injection of turpentine, the accumulation of [^{18}F]FDG was 21.2%, while the accumulation of 2-[^{125}I]-iodo-L-tyrosine reached only 8% [90]. Studies in experimental animals with (*O*-2-[^{18}F]fluoroethyl)tyrosine) [^{18}F]FET showed that unlike [^{18}F]FDG and L-[^{11}C]MET, accumulation of fluorinated tyrosine analog in nonmalignant cells and inflamed lymph nodes was extremely negligible [91].

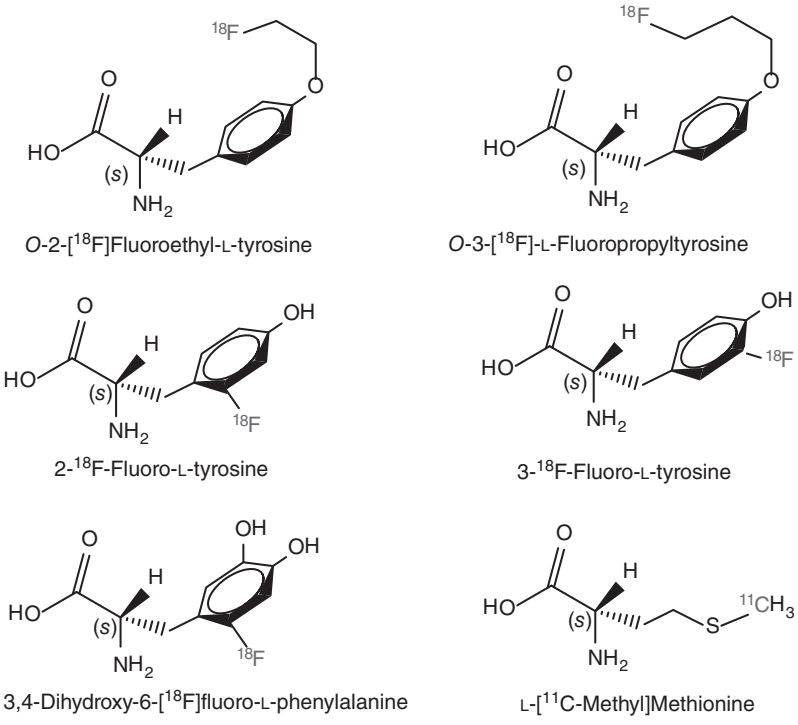


Figure 1.4 PET radiotracers based on α-amino acids.

We can hope that the use of fluorine-labeled amino acids would solve a huge problem in PET diagnostics – the inability to differentiate tumor and inflammation process in the brain by the conventionally used [^{18}F]FDG [92].

The most well-known radiopharmaceutical based on fluorine-labeled amino acids is the 3,4-dihydroxy-6-[^{18}F]fluoro-L-phenylalanine (6-[^{18}F]-L-DOPA) – a fluorinated analog of L-DOPA, the metabolic precursor of dopamine neurotransmitter (see Figure 1.4).

Pharmacokinetic behavior of 6-[^{18}F]-L-DOPA coincides with the behavior of the natural metabolite, which enables a quantitative assessment of dopaminergic neuron density by PET method in the diagnosis and selection of treatment of Parkinson's disease and other disorders associated with a lesion of the dopaminergic system [93]. Recently, the possibility of using 6-[^{18}F]-L-DOPA in the diagnosis of tumors of the gastrointestinal tract and of some other organs was reported [94].

Fluorinated analogs of tyrosine containing fluorine-18 in both the aromatic ring (2-^{18}F-fluoro-L-tyrosine (2-^{18}F-FTYR) and 3-^{18}F-fluoro-L-α-methyl-tyrosine (3-^{18}F-FAMT), and in the side chain – ^{18}F-FET, *O*-3-[^{18}F]-fluoropropyl-L-tyrosine (^{18}F-FPT) [95, 96], are being intensively investigated in a number of PET centers in Europe, Japan, and United States as promising radiopharmaceuticals for PET oncology (see Figure 1.4).

2-[^{18}F]FTYR was among the first amino acids used in PET diagnostics as far back as in 1989 [97]. However, the lack of methods for the synthesis of this RPD in acceptable PET amounts prevents its introduction into clinical practice.

The comparative study of 2-^{18}F-FTYR and L-[^{11}Cmethyl]methionine ([$^{11}CH_3$]MET) in rats with F98 [98] glioma showed similarity in biodistribution and accumulation of 2-[^{18}F]FTYR and L-[$^{11}CH_3$]MET in tumors, indicating the prospectivity of using 2-[^{18}F]FTYR in studies traditionally performed with L-[$^{11}CH_3$]MET.

The study of metabolism and biodistribution of 2-[^{18}F]FTYR in organs of healthy mice [99] as well as human [100] PET studies have shown that the metabolism of 2-[^{18}F]FTYR is characterized by a simpler mechanism as compared with L-[$^{11}CH_3$]MET, the molecule of which rapidly loses radiolabel in the demethylation reaction. Application of 2-^{18}F-FTYR in PET is intensively conducted in many PET centers [100], and the interest in this RPD and other tyrosine derivatives is growing fast.

In Russia, radiopharmaceuticals based on ^{18}F-amino acids have not yet been introduced into clinical practice. Their development and clinical evaluation are highly relevant to expand the possibilities of the method and its implementation in practice of nuclear medicine. The Institute of Human Brain RAS accumulated vast experience of L-[^{11}C]MET used in clinical diagnostics in addition to the [^{18}F]FDG. The development of 2-[^{18}F]-FTYR as a new drug for the diagnosis of tumors by PET is being conducted.

Since nonprotein α-amino acids are physiologically active and suitable for incorporation into pharmaceuticals and for the use as PET radiotracers only in the form of optically active enantiomers, development of methods for their preparation as such is a very important problem of bioorganic chemistry and biotechnology. Unfortunately, traditional microbiological and enzymatic methods for the synthesis of α-amino acids, including chemical synthesis of racemates with their subsequent enzymatic cleavage, are of little use for producing optically active nonprotein α-amino acids because of unusual structures of substrates. Therefore, the asymmetric synthesis is the most applicable approach for the production of such compounds.

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