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Natural and synthetic cytokinins and their applications in biotechnology, agrochemistry and medicine

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The review is devoted to cytokinins — classical plant hormones known for more than six decades. Nevertheless, different aspects of the action of cytokinins are still being investigated. Relevant studies produced interesting, often unexpected, results, which cast doubt on the old paradigms and open new prospects for the use of these phytohormones. Particular attention is given to recent advances in the applications of natural cytokinins and their synthetic analogues in biotechnology, agriculture, medicine and cosmetics. The chemical synthesis, properties and the possible use of artificial cytokinins are considered in detail. The review is aimed at researchers interested in the development and applications of new biologically active compounds with a wide spectrum of action on diverse biological objects, from plants to humans.
The bibliography includes 233 references.

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1. Introduction

In the 1950s, Skoog and co-workers [1] discovered a new class of plant hormones, cytokinins (CKs), which by themselves or together with other classes of phytohormones (primarily with auxins) regulate fundamental biological processes in plants. Subsequent studies provided detailed information on the influence of exogenous CKs on physiological processes in plant tissues.[2–5] Together with auxin, CKs promote the cell division in the shoot apical meristem † and meristems of axillary buds and cambia, but inhibit activity of plant root meristems. Cytokinins affect the main leaf parameters. Thus, they stimulate the leaf growth *via* cell expansion, enhance photosynthesis through the promotion of chloroplast differentiation and stomatal opening and delay senescence. Besides, CKs regulate the ability of organs and tissues to take up various nutrients, activate the pigment formation in cells and stimulate the formation of shoots in undifferentiated tissue culture.

With the onset of the post-genomics era, the range of roles of CKs in plants has been significantly expanded.[6–8] These roles include the regulation of shade avoidance responses, circadian rhythms, the uptake of essential elements (N, P, S, Fe) from the soil, the formation of the vascular system and nitrogen-fixing nodules on plant roots and the female gametophyte development. Cytokinins play an important role in plant defense against environmental stresses of both abiotic (cold, drought, heat, soil salinity) and biotic (viruses, bacteria, fungi) nature. Therefore, CKs are essential for normal development of virtually all stages of the plant life cycle under various living conditions.

The review presents the current state of investigations and applications of CKs and summarizes recent advances in these fields. A distinctive feature of the review is that it covers a broad scope of issues associated with CKs. These issues include the molecular mechanism of action of CKs, their applications in biotechnology, crops, medicine and cosmetics and the chemical synthesis of non-natural CKs and their derivatives. The part of the review devoted to synthetic CKs, their synthesis and prospects of their use has no direct analogues in scientific literature. This is the first review that considers comprehensive literature data and provides an in-depth analysis in this field of science. Previous reviews on this rapidly emerging field of research are more specialized. The latest comprehensive reviews on related issues [9–12] were published in 2016–2017 or earlier and are at present a bit outdated.

The following abbreviations and designations are used in the review:

AHK — *Arabidopsis* histidine kinases, cytokinin receptors,

Asp — D, aspartate,

ATP — adenosine triphosphate,

BA — N^6- benzyladenine (N^6-benzylaminopurine),

BAR — N^6-benzyladenine riboside, N^6-benzyladenosine,

Cbz — benzyloxycarbonyl,

CDI — 1,1′-carbonyldiimidazole,

CHASE — cyclase/histidine kinase-associated sensory (extracellular) domain that is present in transmembrane receptor proteins and is responsible for cytokinin recognition and binding

CKs — cytokinins,

CKX — CK oxidase/dehydrogenase enzymes catalyzing cytokinin catabolism,

Cy — cyclohexyl,

*c*Z — *cis*-zeatin,

DZ — dihydrozeatin,

His — H, histidine,

IC_{50} — half-maximal inhibitory concentration,

iP — Δ^2-isopentenyladenine,

iPR — Δ^2-isopentenyladenine riboside, N^6-isopentenyladenosine,

IPT — isopentenyl transferase, a key enzyme of the CK synthesis,

Kin — kinetin,

KinR — kinetin riboside,

LOG — *LONELY GUY* proteins with 5′-monophosphate phosphoribohydrolase activity, produce active CKs from appropriate nucleoside monophosphates,

MSP — multistep phosphorelay,

Naph — naphthyl,

† Meristems or forming (meristematic) tissues is a general term for plant tissues consisting of rapidly dividing cells that retain physiological activity throughout their life span, ensure the continued growth of the mass of the plant and provide material for the production of various specialized tissues.

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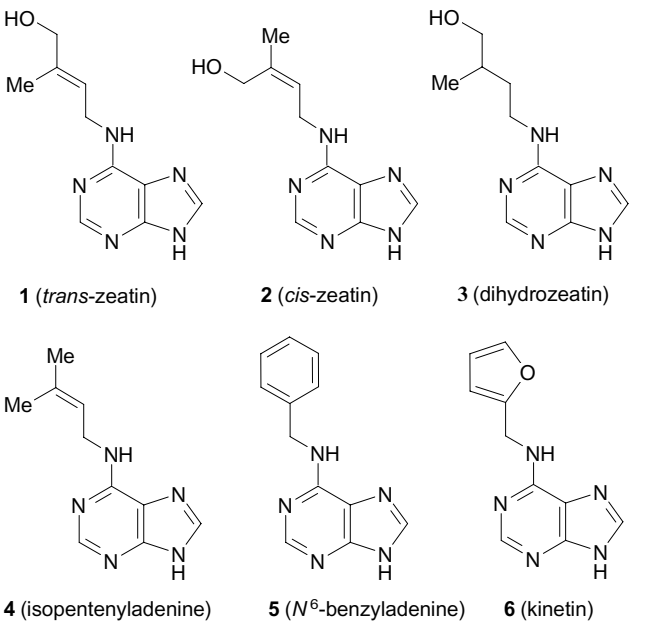
Translation: T.N.Safonova

PhthN — phthalimido,
Py — pyridyl,
QSAR — quantitative structure–activity relationship,
RR — response regulators,
RR-A — type-A response regulators, inhibitors of cytokinin signalling,
RR-B — type-B response regulators, transcription factors,
STAT — signal transducer and activator of transcription,
TD — *N*-phenyl-*N*′-1,2,3-thiadiazol-5-ylurea (thidiazuron),
*t*Z — *trans*-zeatin,
*t*ZR — *trans*-zeatin riboside.

2. Cytokinins as classical plant hormones

N^6-Substituted adenine derivatives are natural CKs. The isoprenoid chain and its derivatives and also an aromatic ring (simple or modified) can serve as substituents on the exocyclic nitrogen atom.[3–5,7] The commonly known CK derivatives of adenine (structures **1**–**6**) are shown below.

Structures 1–6



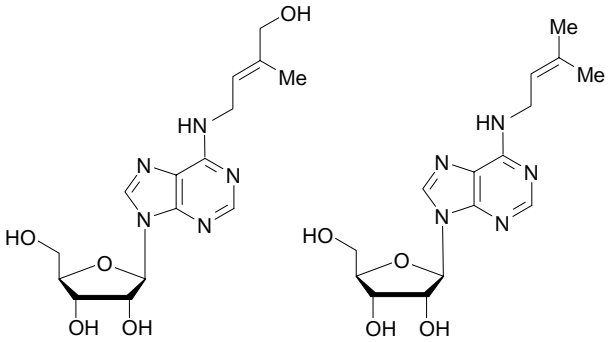
Hormonal activity of CKs depends on their structure. *trans*-Zeatin (**1**, *t*Z) is the most known naturally occurring CK. It exhibits high affinity for all the tested isoforms of CK receptors with an apparent constant *K*d in the range of 1–10 nmol L^{-1}.[7,13–15] Apart from *t*Z, natural CKs are *cis*-zeatin (**2**, *c*Z), dihydrozeatin (**3**, DZ), isopentenyladenine (**4**, iP) and adenine derivatives containing a phenyl [**5**, N^6-benzyladenine (BA)] or oxyphenyl (topolins) side chain at the N(6) position.

cis-Zeatin (**2**) shows much weaker cytokinin activity compared to the *trans* isomer and other natural CKs. Nevertheless, the function of the *cis* isomer may be to maintain certain physiological processes in plants, particularly under adverse environmental conditions.[16] As opposed to other CKs, *c*Z (**2**) is derived mainly from the hydrolysis of transfer RNA (tRNA).[3,5,7,17,18]

Like other typical hormones, CKs act in plants at very low concentrations, mainly at a nanomolar level (1–50 pmol g^{-1} of fresh weight).[3,5,19] It should be borne in mind that the plant response to CKs depends on the CK concentration, and at high CK levels the response can be reversed.[3,20] Cytokinins can act as short-distance (paracrine) regulators of the plant growth and development, *i.e.*, at the site of their formation, and as autocrine regulators through long-distance transport along the xylem (the main water-conducting tissue) from the root to shoots and(or) in the opposite direction *via* the phloem tissue that translocates products of photosynthesis.[3,6,21,22] The CK composition varies in different tissues. Thus, *t*Z (**1**) and particularly *trans*-zeatin riboside (**7**, *t*ZR) predominate in the xylem, whereas iP-type derivatives prevail in the phloem (**4**, **8**).[23–25]

Cytokinin biosynthesis and metabolism. Cytokinin biosynthesis is catalyzed by isopentenyl transferases (IPT) and

Structures 7, 8



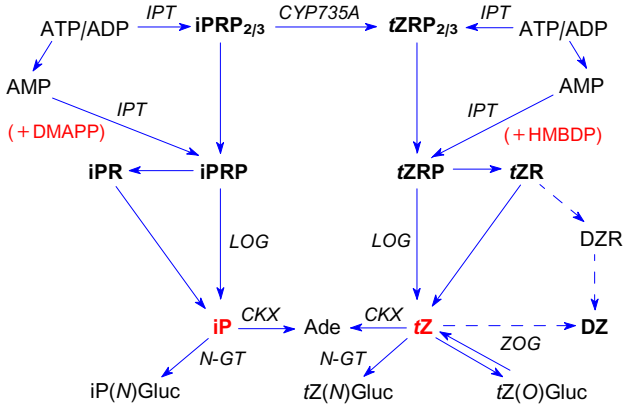


Figure 1. Key biosynthetic and metabolic pathways of the active cytokinins Δ^2-isopentenyladenine (iP) and *trans*-zeatin (*t*Z) and also dihydrozeatin (DZ). ATP/ADP is adenosine tri/diphosphate; AMP is adenosine monophosphate; iPR, *t*ZR, DZR are cytokinin ribosides; iPRP, *t*ZRP are cytokinin ribotides; Ade is adenine; iP(*N*)Gluc, *t*Z(*N*)Gluc, *t*Z(*O*)Gluc are CK glucoconjugates. DMAPP is dimethylallyl diphosphate; HMBDP is a hydroxylated derivative of DMAPP. Enzymes are indicated in italics: CYP735A is cytochrome P 735A; CKX are CK oxidase/dehydrogenase enzymes catalyzing cytokinin catabolism; IPT are isopentenyl transferases, key enzymes of the CK synthesis; LOG are *LONELY GUY* enzymes with 5′-monophosphate phosphoribohydrolase activity, produce active CKs from appropriate nucleoside monophosphates. The scheme is composed using the data published in the literature.[4,18,19,25]

5′-monophosphate phosphoribohydrolases (LOG); the reversible and irreversible CK inactivation is mediated by cytokinin *O*- and *N*-glycosyltransferases (ZOG and N-GT), respectively; the CK degradation is promoted by cytokinin oxidases/dehydrogenases (CKX) (Fig. 1). These processes and relevant enzymes are described in detail in reviews.[4, 18, 19, 25]

3. Mechanism of action of cytokinins

The signal transduction occurs *via* the CK binding to catalytic receptors with histidine kinase activity. Three isoforms of CK-binding histidine kinases were identified in the model plant *Arabidopsis thaliana*: AHK4/CRE1/WOL (*Arabidopsis Histidine Kinase*, *Cytokinin Response 1*, *Wooden Leg*, respectively),[26, 27] AHK2 and AHK3.[28–30] Cytokinin receptors are large multidomain transmembrane proteins located mainly in the endoplasmic reticulum and, apparently, at the plasma membrane of the cell.[22, 31–36] The N-terminus of the receptor molecule bears the CHASE (cyclase/histidine kinase-associated sensory) domain[37–39] containing a single ligand-binding site in the PAS subdomain[39–42] (Fig. 2). Protein domains with catalytic and phosphate-acceptor activity are located on the cytosol side of the cell.[7, 33, 42]

According to the modern concept, the activated receptor exists as a dimer, two subunits of which with CK-induced histidine kinase activity catalyze the phosphorylation of each other at the conserved histidine (His) residue.[43–45] The C-terminus of the receptor bears the acceptor domain containing the conserved aspartate (Asp) residue, which accepts phosphate from phosphohistidine. This Asp residue is a kind of phosphoryl group carriers from the receptor to the soluble phosphotransmitter protein (HPt), which transfers 'hot' phosphate to the nucleus of the cell.[7, 33, 46]

The cytokinin signal is transduced from the receptor to cytokinin primary response genes *via* the so-called multistep phosphorelay (MSP) pathway. Apart from the receptor and the phosphotransmitter, nuclear response regulators (RRs)

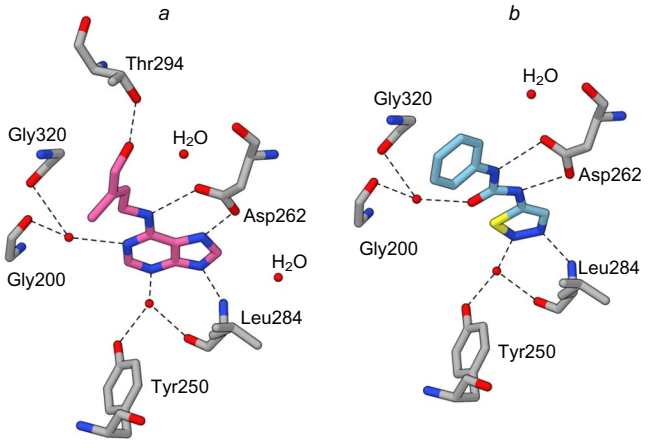


Figure 2. Key protein–ligand interactions of the natural cytokinin *trans*-zeatin (**1**) (*a*) and the synthetic cytokinin thidiazuron (**9**) (*b*) in the hormone-biding site of the AHK4 receptor. The Figure clearly illustrates the similarity of the specific binding determinants of the natural and synthetic ligands. The Figure was drawn using the crystal structures of the AHK4 receptor in complexes with cytokinins (PDB ID: 3T4L and 3T4T).

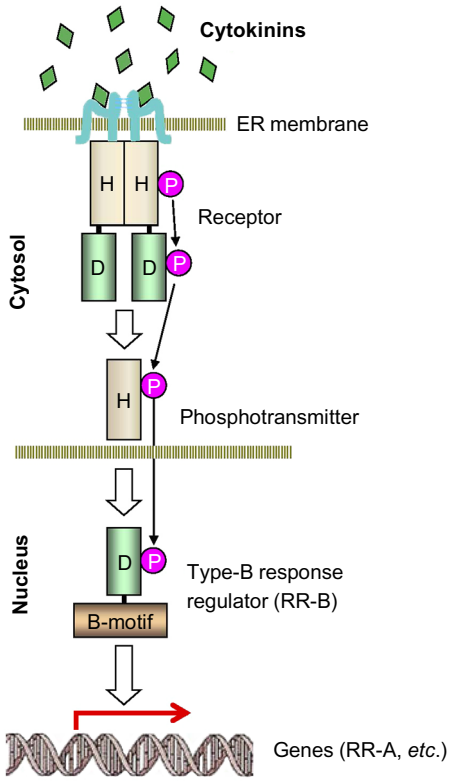


Figure 3. Main cytokinin signal transduction pathway in the plant cell: from the receptor dimer incorporated in the membrane to primary CK response genes.[22] ER is the endoplasmic reticulum; RR-A and RR-B are type-A and type-B response regulators, respectively; P is a phosphate residue that is transferred from the cytosol to the nucleus;[22] D and H are conserved phosphorylated aspartate and histidine residues, respectively.

are involved in the MSP pathway (Fig. 3). Two types of RRs (A and B) are known. Type-B response regulators (RRs-B) act as transcription factors and regulate the activity of cytokinin primary response genes, recognizing the short consensus sequences (A,G)GAT(C,T) in promoters of these genes. Type-A response regulators (RRs-A) are homologous to RRs-B, but they do not act as transcription factors because they lack the DNA-binding domain. Type-B response regulators are activated *via* the phosphorylation of the conserved Asp residue in their structure followed by their interaction with responsive promoters, generally activating the transcription of a certain set of genes. Type-A response regulators are products of cytokinin primary response genes. Their synthesis is rapidly initiated in response to CKs, and RRs-A begin to capture phosphates needed for the activation of RRs-B. This underlies the negative feedback mechanism, which is typical of not only CKs but other plant hormones.[3, 7]

Therefore, the signal transduction triggered by cytokinins occurs *via* a phosphorylation–dephosphorylation His-Asp-His-Asp relay mechanism (see Fig. 3). This process is blocked through dephosphorylation of phosphotransferases by phosphatase; in the absence of CKs, the CRE1/AHK4 receptor acts as phosphatase.[47] The *Arabidopsis* protein AHP6 belongs to so-called pseudo-phosphotransmitters. This protein does not contain the conserved histidine residue required for the phosphorylation and generally competes with typical phosphotransmitters for the binding

to the receptor or the response regulator, thus inhibiting CK signalling.[48]

Although CKs were discovered as phytohormones that regulate a wide range of biological processes in plants, including their growth, differentiation, productivity and senescence, they were found to have more diverse functions. Currently, CKs are also of interest in terms of their effects on animal and human cells and organisms. It is worth noting that current knowledge of cytokinin signalling in plants suggests that there is a fundamental difference between the mechanisms of action of CKs in plant and animal cells.

4. Applications of cytokinins

4.1. Applications in crop production

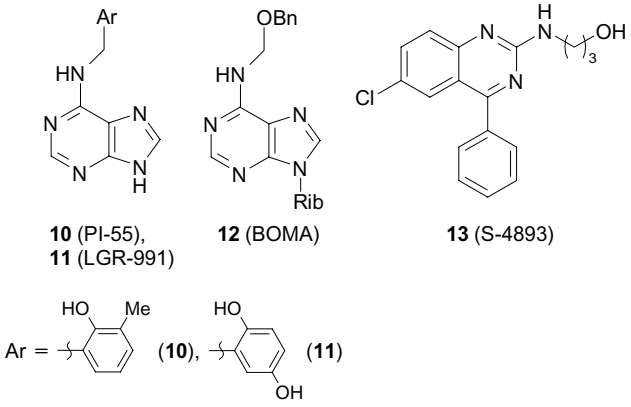
As mentioned above, CKs influence various parameters of the plant growth and development throughout the ontogenesis, primarily regulating the cell proliferation and differentiation.[2–5,49] Cytokinins have a positive effect on the growth of the above-ground part of plants stimulating the activity of apical and lateral meristems and cambia.[50] The cytokinin deficiency in plants reduces the chlorophyll content.[51]

At first sight, the effect of CKs on the root system is controversial. The plant roots, in which the cytokinin signalling is suppressed for some reason, show faster growth and development compared to wild-type roots and become longer and more branched.[50,51] Exogenous CKs inhibit root elongation due to a decrease in the number of dividing cells and the root meristem size.[52] However, triple mutants for cytokinin receptors of *Arabidopsis*, whose cells lose the capability of CK signal perception, are characterized by a significant reduction of the root system.[51,53] In explaining this seeming paradox, researchers stem from the postulate that the functions of CKs in roots are fundamentally similar to those in shoots. Exogenous CKs inhibit the wild-type root growth due to the supraoptimal level of endogenous CKs in roots.[53,54] Therefore, a moderate decrease in the CK signalling in roots to the optimal level stimulates the root growth. However, a further decrease in signalling and all the more the complete loss of CK receptors, on the contrary, have an adverse effect on the root development. The described effect of CKs on root systems has stimulated the design of new plant growth regulators. For example, anticytokinins (antiCKs) exert a positive effect on the root development.

Initially, the cytokinin activity of different classes of compounds was evaluated using classical bioassays, which are most often based on the stimulation of tobacco callus growth, stabilization of the chlorophyll content of cereal leaves and the dark induction of betacyanin biosynthesis in *Amaranthus* cotyledons.[55] In recent years, classical bioassays have been increasingly replaced by their state-of-the-art analogues. Modern highly specific and high-throughput assay systems are based on the quantitative evaluation of activity of CK-responsive promoters (*e.g.*, the *ARR5* gene promoter from *Arabidopsis*) fused to the reporter gene (*GUS* gene) or based on the expression of single cytokinin receptor genes in transformed cells followed by the examination of ligand–receptor interactions.[10,56–59] In the former case, the cytokinin activity of the compound is revealed and evaluated; in the latter case, the affinity of the compound to the cytokinin receptor is assessed. In both cases, individual receptors can be tested, which provides new ways to detect unique compounds with specific cytokinin or anticytokinin activity. In general, a distinct correlation between the ligand binding and hormonal activity was established for *Arabidopsis* receptors; the correlation coefficient is in the range of 0.8–0.9.[58]

In the past decade, the screening of hundreds of synthetic analogues of naturally occurring CKs revealed real antiCKs. The mechanism of action of CK antagonists may rely on both the competitive [compounds PI-55 (**10**), LGR-991 (**11**), BOMA (**12**)][60–62] and non-competitive [S-4893 (**13**)][63] inhibition of CK binding to receptors. It is interesting that competitive inhibitors of cytokinins are purine derivatives, whereas non-competitive inhibitor **13** (S-4893) shows no similarity to CKs, having a 4-phenylquinazoline structure.

Structures 10–13



10 (PI-55), **11** (LGR-991) **12** (BOMA) **13** (S-4893)

Besides, growth regulators, receptor agonists, can be designed based on synthetic CKs, which can selectively interact with individual receptors and, consequently, act preferably on the plant organ, where these receptors are predominantly expressed.[58]

Cytokinins affect not only the root growth but also root functioning: the uptake of essential elements, like N, P, S and Fe, from the soil and the formation of nitrogen-fixing nodules (in legumes). The treatment of nitrogen-fixing bacteria with exogenous CKs activates early nodulin genes, which are upregulated upon plant infection and nodule formation.[6,8,64] It should be noted that the action of CKs on the nodulation depends on diverse factors.[65]

Cytokinins play an essential role in the nitrogen metabolism regulation. Cytokinin metabolism in plants is closely related to nitrogen availability and metabolism,[66] thus correlating with a high nitrogen content in CKs themselves. The leaf growth is stimulated by NO_3^- ions and is accompanied by an increase in the CK concentration in the xylem sap and in the total green matter.[23,67] The uptake of such root CKs improves the photosynthesis and nitrogen distribution in shoots,[66] resulting in greater efficiency of nitrogen fertilization and higher crop yields.[68]

Cytokinins, like other phytohormones, play critical roles in helping the plants to adapt to adverse environmental conditions.[69] Abiotic stresses (drought, cold, heat and salinity) result in an altered CK distribution pattern in the plant body due primarily to an increase or decrease in the activity of CKX enzymes, which catalyze the CK degradation.[70,71] In tobacco plants under drought stress conditions, CKs reduce the oxidative damage by reactive oxygen

species, although there is evidence that plants with disrupted CK signalling show enhanced tolerance to photooxidative stress.[69,72]

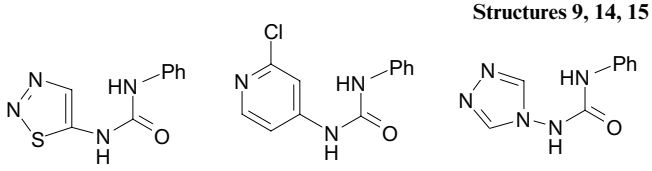
It is assumed that the action of CKs also depends on the stress intensity. Under moderate stress conditions, stabilized or elevated CK levels maintain the plant growth, whereas a decrease in the CK level in response to strong abiotic stress conditions promotes the mobilization of all resources to ensure the survival of plants.[73] As a result, once the stress factor is removed, plants with stabilized or elevated CK levels return to homeostasis much faster than plants in which CK levels are significantly decreased during stress.[74] Besides, CKs protect the photosynthetic apparatus under high light.[72,75] Cytokinins exhibit the protective effect due to an increase in the activity of antiooxidant enzymes. All these data provided the basis for the use of CKs and their analogues as plant growth regulators with antistress properties.[75,76] However, cytokinins were found to exert both positive and negative effects on the stress resistance of plants depending on the plant species and particular growth conditions.[75,77] The appropriate biotechnological manipulation of CK contents can lead to a dramatic increase in the survival and productivity of plants under adverse conditions,[76] which makes it possible to increase the yield of high-value crops. The reduced expression of CKX was found to increase the grain yields of rice[78] and barley[79] due to an increase in the amount of seeds per plant and the average seed biomass. Similarly, the overexpression of the OsABCG18 protein, which exports CKs from roots to shoots, led to a considerable increase in the CK content in shoots and in the rice grain yield.[80]

In agricultural practice, plants (fruit trees, vegetables and ornamental plants) are treated with exogenous CKs to accelerate the plant growth, stimulate the germination and lateral branching and, finally, improve yield traits.[81] Various CKs and CK-based substances were tested in order to develop approaches to their large-scale applications.[82] The tested plants (in particular, in field conditions) included cereals (wheat, barley, rice, maize, oat, sorghum, *etc.*), legumes (soybean, faba bean, pea, *etc.*), vegetables (tomato, lettuce, eggplant, *etc.*), cotton and fruit trees (apple tree, pear tree, kiwi, *etc.*). The tests were performed using CKs, such as adenine derivatives [mainly, the stable compounds BA (**5**) and Kin (**6**), often as mixtures with other phytohormones] and, in some cases, phenylurea derivatives, such as thidiazuron **9** (TD) or **14** (CPPU, or 4PU-30).

Positive effects induced by CK application were observed in most cases. In cereals, an increase in the grain yield was achieved, particularly under stress conditions; in vegetables, the CK application stimulated the plant growth; in fruit trees, the fruit size and weight were increased. A special case is cotton, whose treatment with the adenine derivatives BA (**5**) and Kin (**6**) improved the yield and enhanced drought resistance, while the treatment with the phenylurea derivative TD (**9**) caused plant defoliation[82,83] required for mechanical harvesting. It is worth noting that TD (**9**) has gained particular interest of researchers due to its dual role. On the one hand, it acts as an efficient receptor agonist *via* the canonical signalling pathway; on the other hand, it serves as an inhibitor of CKX enzymes, increasing the content of endogenous CKs.[84] Yet another beneficial effect of exogenous CKs is due to their ability to decrease the toxicity of pesticides, which are used to treat cultivars, as a result of an increase in the activity of antioxidant enzymes. This effect was observed, in particular, on treatment of tobacco plants[85] and rye[20] with the pesticide paraquat in combination with CKs.

In Russia, the phenylurea derivative, *N*-(1,2,4-triazol-4-yl)-*N*′-phenylurea, with strong cytokinin activity is produced under the trademark Tsitodef (**15**) for use in agriculture and floriculture.[20] A 4% Tsitodef solution was recommended for use on fruit trees to increase the fruit yield and enhance the post-harvest storage life, on vegetables to increase the product yield, on sugar beet to enhance the sweetness of root crops, on ornamental plants to increase the colour intensity and the size of flowers. The rules and regulations were specified for the use of compound **15** on onion, garlic, grape, cucumber, potato, cereals, apple tree and cotton plants.

Structures 9, 14, 15



9 (Thidiazuron) **14** (CPPU) **15** (Tsitodef)

An important role of CKs is to regulate plant immunity during host–pathogen interactions. Some pathogens exploit the cytokinin pathway of plants either through direct synthesis of CKs or by altering the cytokinin signalling in plants. For example, the parasitic bacteria *Agrobacterium tumefaciens* contain genes for the biosynthesis of CKs (and auxins) in the Ti plasmid. This allows them to locally control plant organogenesis and induce gall tumours.[86] The bacteria *Rhodococcus fascians* secrete an array of CKs, thereby causing the proliferation of young shoot tissues and growth of leaf galls.[87] On the other hand, the treatment of plants with exogenous CKs or an induced increase in the level of endogenous CKs mediates plant resistance against infections with many different types of pathogens.[86] For instance, the use of Kin for the treatment of tobacco leaves infected with *Pseudomonas syringae* leads to a significant decrease of leaf spotting.[88] Transgenic *Arabidopsis* plants having stabilized CK levels exhibited enhanced resistance against infection with the pathogen *Verticillium longisporum*.[89] The broad protective effect of CKs is greatly attributed to the fact that they trigger salicylic acid (SA) signalling giving rise to induced systemic resistance of plants against pathogenic infections.[86,90]

4.2. Applications in biotechnology

Cytokinins are essential components of artificial plant tissue culture media. They are widely used in working with plant tissue and cell cultures, in the micropropagation and transgenic plant production,[3] where both natural CKs and their synthetic analogues are applied.[10] The discovery of cytokinins was due to their ability to induce cell division in cultures.[1,91] Subsequent studies showed that the addition of CKs to culture media causes shoot organogenesis from callus, but at the expense of root development.[92]

Cytokinins affect the in vitro plant morphogenesis in different ways depending on the molecular structure and the concentration of the compound. All types of cytokinins can induce the shoot formation, but the most suitable CK and the most efficient concentration largely depend on the plant species.[93] This is due to the fact that different species and even varieties of plants differ with respect to the uptake,

transport, perception, metabolism and level of endogenous CKs produced in the explant.[94] At high CK concentrations ($>5-10$ mg L^{-1}) in the culture medium, the efficiency of morphogenesis is reduced down to its complete blockage regardless of the plant species, types of the explant and CK.[95] In most of procedures for plant transformation, their regeneration in tissue cultures *via de novo* shoot organogenesis is a critical stage. Methods of the efficient *in vitro* shoot regeneration and micropropagation using various CKs at different concentrations were developed for many plant species, including potato plants, sugar cane, banana, apple tree, strawberry and many medicinal and ornamental plants.[10,96] Currently, plant secondary metabolites are produced on an industrial scale and are used as pharmaceuticals, agrochemicals, food flavourings, colouring agents, pesticides and food additives. These biotechnologies utilize plant cell and organ cultures, which cannot be established and propagated without plant hormones, in particular CKs.[97] Cytokinins were identified as modulators of secondary metabolism in cultured plant cells. The absence of CKs in media often leads to chlorophyll loss in cells, necrosis and a considerable decrease in the secondary metabolite content.[98] In some cases, the addition of CKs can not only maintain the yield of useful products at a certain level but lead to a significant increase in the crop yield. The addition of BA (**5**) was shown to improve the production of saponins in ginseng, and iP (**4**) stimulated the production of artemisinin in annual wormwood (*Artemisia annua* L.).[97] In the case of micropropagation of other valuable plants rich in secondary metabolites, the correct choice of cytokinin additives enhances the production of secondary metabolites by daughter plants.[99]

Besides, CKs are applied to induce and(or) enhance the *in vitro* tuberization in potato plants. This method is employed to produce seed tubers as planting material for potato harvest. The addition of $1-5$ mg L^{-1} CKs to the medium promotes microtuberization in normally propagated plants.[100–102]

Some applications of cytokinins and(or) related genes in biotechnology were patented. Examples are applications of CKs for the enhancement of shoot growth on trees[103,104] and the *in vitro* propagation and transformation of medicinal plants.[105] An alternative approach is based on the use of certain cytokinin-regulated genes. For instance, IPT transgenes of CK synthesis are applied to enhance the plant growth and increase the plant productivity,[106] in particular, to increase the biomass of valuable tree species.[107] Similar practical applications involve *CKX* genes,[104,108] AHK receptor genes,[109] *LOG* genes[110,111] *etc.* These far from complete applications demonstrate significant promise of new methods of homeostasis modulation and(or) CK signalling in biotechnology of plants.

4.3. Applications in medicine

In recent years, various assays of CKs and their analogues were performed in order to evaluate the possibility of using these compounds in medicine as antitumour, antiviral, antiparasitic and other pharmaceutical agents and as inhibitors of neurodegeneration. It should be noted that CKs are the only typical phytohormones, which show promise as drugs.

The key role of CKs in the regulation of plant cell growth and differentiation suggests that such compounds can exert a significant effect on biochemical processes in animal and human cells. This assumption was confirmed, although CKs and their derivatives (ribosides, ribotides) affect animal and plant cells in different ways.

For instances, CK ribosides exhibit more or less efficient cytotoxicity against a wide range of human malignant cells: glioblastoma, rhabdomyosarcoma, breast cancer, central nervous system (CNS) tumours, colorectal cancer, lung cancer, leukaemia, melanoma, prostate, ovarian and kidney cancers.[112–114] *ortho*-Topolin riboside (**16**, *o*TR) was found to have high activity ($IC_{50} = 0.5-11.6$ μmol L^{-1}) against some human tumour cell lines. The corresponding nucleotides exhibited comparable activity, while free bases were inactive.[113] This is a considerable difference between the action of CKs in animal and plant cells. Thus, CK bases rather than ribosides are active in plant cells.[114] This fact suggests the existence of different mechanisms of action of CK-like compounds on plant and animal (human) cells. Actually, animal cells lack sensor histidine kinases and multistep phosphorelay signalling systems responsible for cytokinin signalling in plants. Nevertheless, it was reported that the cytokinin activity of compounds in plant cells correlates with their cytotoxic activity in human cancer cells.[115]

Investigations of N^6-isopentenyladenosine (**8**, iPR), N^6-benzyladenosine (**18**, BAR) and their analogues in several cancer cell lines demonstrated that the presence of ribofuranose is a necessary condition for these compounds to exhibit cytostatic properties. The replacement of this substituent by another moiety led to the loss of anticancer activity. The type of cancer cells sensitive to a particular compound is dictated by the structure of the side chain at the N^6 position of the purine heterocycle.[9]

The majority of clinical trials on the use of CK ribosides in anticancer therapy were performed with iPR (**8**) because it was the first CK riboside whose anticancer activity was found in the late 1960s. Considerable attention has also been given to BAR (**18**), a rather inexpensive stable compound.[12] However, agents based on CK ribosides were not registered as drug products.[116] Evidently, the reason was that effective CK ribosides were toxic not only to cancer cells but also to normal tissues. Clinical trials of iPR and BAR performed in the mid-1970s in patients with different cancer types showed that agents based on these derivatives have a low therapeutic index. The administered dose of the compounds was limited by their toxicity (primarily hepatotoxicity), and injections were discontinued, although the improvement of the state (remission) was observed in some cancer patients.[117] The compound ZR (**7**), which is much less toxic compared to other CK ribosides, could be a suitable drug candidate.[118] However, the efficacy of ZR as an anticancer agent is also low,[113] and its application remains an open question. Hence, there is a need to search for new compounds (ribosides), which will induce selective damage of cancer cells without affecting normal ones. The promise of these efforts is evidenced by the ability of some compounds to induce in cancer cells their own systems (genes) associated with anticancer protection (tumour repressors). Therefore, the elucidation of the mechanism of antitumour action of CK ribosides is to date a topical task.

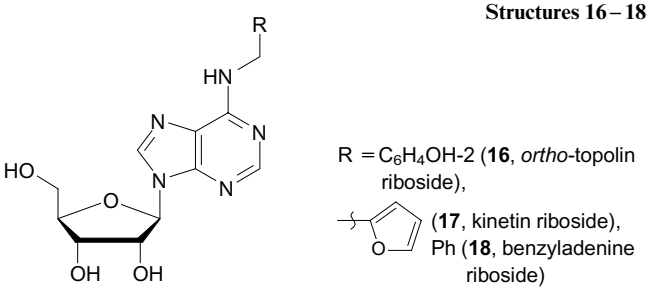
The activity of CK ribosides is known to be mediated by apoptosis induction or cell cycle blockage in cancer cells.[119] Cytokinin ribosides generally induce apoptosis in cell lines derived from hematologic malignancies. The treatment of solid tumour cells with CK ribosides can induce both apoptosis and disruption of different mitosis phases depending on the cell line and the riboside used.[12] Kinetin

riboside (**17**, KinR) was considered as the active substance for the treatment of human myeloma cancers and other types of malignancies (lymphoma, carcinoma, melanoma). Experiments in mice showed that compound **17** selectively suppressed the transcription of genes for the biosynthesis of cyclins D1 and D2, resulting in the arrest of the cell cycle and selective apoptosis of tumour cells.[120] Unfortunately, subsequent studies showed that the treatment of cancer cells with KinR causes undesirable resistance (apparently, due to the inhibition of cyclin D1 biosynthesis) against the commonly used anticancer drug Gefitinib.[121]

Meanwhile, CK ribosides can have a wider range of action. In particular, KinR (**17**) can block multiple oncogenic pathways by initiating the biosynthesis of tumour repressors.[120] It is assumed that animal cells recognize CK ribosides *via* the adenosine $A_{2A}R$ and(or) A_3R receptors followed by activation of protein kinase A and the alteration of adenylate cyclase activity.[121, 122] This is accompanied by a decrease in the adenosine triphosphate (ATP) level in cells, but leads to accumulation of CK nucleotides and a simultaneous change in the content of pro- and antiapoptotic proteins,[12] ultimately resulting in cell apoptosis. Besides, adenosine receptors trigger various physiological and pathological processes. For instance, the $A_{2A}R$ receptor affects inflammatory processes in tissues and the development of neurodegenerative disorders, in particular Parkinson's disease.[123]

Besides, iPR was shown to be able to form complexes with DNA and induce genes associated with downregulation of the cell cycle.[9] Many CK ribosides, *e.g.*, ZR (**7**), iPR (**8**), *o*TR (**16**), and BAR (**18**), proved to be able to interact with some proteins, which are somehow involved in antitumour activity of cells.[12] In particular, it was demonstrated that *o*TR triggers the mitochondrial apoptotic pathway in cancer cells.[124] The apoptosis is blocked by the adenosine kinase inhibitor (A-134974), which indicates that *ortho*-topolin riboside (*o*TR) (**16**) should be transformed into ribotide for the effect to be manifested. In another study performed in human acute myeloid leukaemia cells, *o*TR was found to alter the activity of a group of phosphorylated proteins, signal transducer and activator of transcription (STAT) proteins, which ultimately results in cell differentiation accompanied by the loss of oncogenicity.[125]

Structures 16–18



16–18

As opposed to CK ribosides, CK bases exert a favourable cytoprotective action on animal cells. This is primarily associated with potent antioxidant properties of CKs. The oxidative stress can damage cells in different ways: *via* DNA, protein and lipid damages or *via* induction of apoptosis and cell death. In experiments in human cell lines, the cells were treated with different oxidants or toxins, which induced oxidative stress, mediated DNA damages and reduced cell viability.[126] The pretreatment of cells with Kin (**6**) at concentrations of up to 100 nmol L^{-1} had a significant protective effect against DNA and cell damage, and the percentage of non-viable cells returned to the control level. These results are consistent with the data of a publication,[127] which showed that Kin inhibits apoptosis of ageing spleen cells induced by D-galactose in rats, and with the evidence that Kin plays a role in protecting cells against oxidative stresses mediated by the Fenton reaction.[128] Kinetin (**6**) can suppress the free radical formation associated with platelet aggregation and appeared to have antithrombotic activity.[129] Oxidative stress reduced the level of glutathione, acting as an endogenous antioxidant in cells, while Kin protects cells against this effect.[126, 130] The maintenance of an appropriate glutathione level may be indicative of the possible mechanism of antioxidant activity of CKs.

The cytokinins BA (**5**) and Kin (**6**) *in vitro* stimulated the activity of antioxidant enzymes in fibroblast cells, which may be used in the prevention and treatment of many skin diseases associated with oxidative stress, such as psoriasis and granuloma annulare.[130–132] Kinetin was shown to have a similar antioxidant effect in thymus tissues and, consequently, Kin can apparently maintain immunity and protect thymus cells against ageing.[127]

Cytokinins can protect cells not only against oxidative stress but also other stresses and prevent some detrimental effects of cell ageing. For example, human skin fibroblasts cultured in the presence of Kin or *trans*-zeatin (**1**) retain some characteristics of cells of lower passage. It is worth noting that experiments with invertebrates showed that Kin is even able to increase their lifespan.[114]

Cytokinins and their ribosides exhibit neuroprotective activity. *trans*-Zeatin (**1**) has a broad spectrum of activity in the treatment of central nervous system diseases, in particular Alzheimer's disease.[133] *trans*-Zeatin is responsible for the inhibition of rat acetylcholinesterase and has beneficial effects on cell viability, reducing the production of reactive oxygen species (ROS). Kinetin protects rat astrocytes *in vitro*, as well as the rat brain against glycoxidative damage in the D-galactose model *via* promotion of antioxidant defense system. *trans*-Zeatin (**1**) showed a beneficial effect in the long-term treatment of scopolamine-induced amnesia.[114] Kinetin and its nucleotide have therapeutic activity against Parkinson's disease. The mechanism of their action is based on the activation of kinase PINK1 in brain cells, the loss-of-function mutations of which leads to early-onset Parkinson's disease.[134, 135] Kinetin has passed clinical trials and was approved for the treatment of the neurodegenerative disease, familial dysautonomia. Kinetin was found to improve aberrant pre-matrix RNA (mRNA) splicing in the *IKBKAP* gene, responsible for this disease, the mechanism of which is still poorly understood.[114] *trans*-Zeatin riboside also has neuroprotective effect and is highly promising for the treatment of neurodegenerative diseases.[122]

It was reported that CKs can exert anti-inflammatory activity.[136–138] It is also of interest that Kin has inhibitory activity against the bacteria *Helicobacter pylori* responsible for stomach ulcer.[139] It was reported that iPR and BAR have antiviral activity.[9] Synthetic BAR derivatives can suppress the development of some protozoal diseases;[9, 140] TD (**9**), a synthetic CK, inhibits the growth of malarial plasmodium.[141]

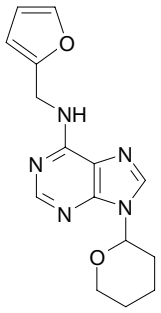
4.4. Applications in cosmetics

The beneficial effect of CKs [*t*Z (**1**), BA (**5**) and Kin (**6**)] on *in vitro* cultured cells, primarily on epithelial cells, was found in early studies.[128, 142] This fact suggested that these phytohormones can be used as topical agents for skin protection and recovery. Subsequent studies of the effects of CKs on epithelial cells were performed in parallel with practical applications of CKs as creams and lotions (see reviews [11, 114]). It was found that, when added to normal (non-malignant) fibroblasts in moderate doses (50–100 μmol L^{-1}), CKs slow down typical signs of ageing such as cell expansion, the appearance of multinucleated cells, accumulation of lipofuscins, hyperpolymerization of actin filaments, *etc.*[11, 118, 143] Regular skin care with a cream containing 2% Kin improved skin hydration and elasticity and made skin resistant to UV light.[144] Ultraviolet rays accelerate skin ageing, primarily causing its dehydration accompanied by the loss of aquaporin (AQP3) acting as a membrane transporter of water. Cytokinin (*t*Z, **1**) prevents the loss of AQP3, preserving the skin structure and hydration and accelerating cutaneous wound healing,[145] which may be one of protective action mechanisms.

Another mechanism of action of CKs is underlined by their ability to prevent the development of oxidative stress and reduce its detrimental effects, as mentioned above. It was demonstrated that Kin and BA enhance activity of antioxidant enzymes in cells, protecting DNA, membrane proteins and phospholipids against oxidation and glycosylation.[130, 146] A study using human keratinocytes in culture confirmed the protective effect of Kin against oxidative stress in cells, but only at low hormone concentrations (below 100 nmol L^{-1}).[126] High hormone concentrations can lead to opposite effects, enhancing oxidative stress and causing cell apoptosis. Apparently, different cell lines have different sensitivity to CKs, which is manifested in the differences in active concentrations of CKs reported by different research groups.

Ointments and lotions containing CKs or their close analogues as an active ingredient are commercially produced and are commonly used by millions of consumers. Creams containing pyratine (**19**), an analogue of Kin (**6**) bearing the tetrahydropyran substituent at the N(9) position, have gained popularity.[147]

Structure 19



19 (Pyratine)

These creams were evaluated in clinical trials and were recommended for middle-aged and elderly people to prevent undesirable age-related changes in skin mechanical properties and UV-induced changes. Besides, pyratine-containing creams and lotions are efficient in the treatment of some skin diseases associated with oxidative stress,[148] as mentioned above. Two synthetic analogues of Kin (**6**), in particular an analogue of pyratine (**19**) containing chlorine at the 2 position of the adenine base, were found to be effective in the protection of skin against UV irradiation. It is worth noting that, although these compounds are not true antioxidants, they can protect cells against oxidative stress.[149]

Therefore, CKs control a wide range of processes, including the plant growth, differentiation and senescence, and affect the proliferation and functions of animal (human) cells. Although, as opposed to plants, animal cells lack a system responsible for the perception of cytokinin signal, there is a certain similarity in the action of CKs in plant and animal cells. Primarily, it is a significant antioxidant effect caused by CK, bases and also the ability to induce cell differentiation. Cytokinins are of particular interest as pharmaceutical and cytoprotective agents. Along with natural CKs, synthetic CKs (thidiazuron, tsitodef, pyratine, *etc.*) are applied in crops, biotechnology, medicine and cosmetics. This suggests the importance of the design and synthesis of new synthetic CKs and CK-like compounds, which can be used in diverse areas of human activity. The relevant issues are considered in subsequent Sections of the review.

5. Synthetic cytokinins and their analogues

Cytokinins are widely used in agrochemical, biotechnological and medicinal preparations. Hence, the search for new synthetic analogues of CKs with higher efficacy and selectivity of action compared to natural compounds has attracted great attention. Synthetic growth regulators are applied due to their high activity and lower cost and toxicity. Besides, they are often metabolically more stable, resistant to oxidases and retain biological activity at low concentrations.[10, 55, 114, 115, 150, 151]

The structures of substituents at nitrogen atoms may vary in a wide range, but they should meet certain requirements.[152] The general strategy for the development of CK analogues includes three main approaches:

— structural modification of natural compounds, the synthesis of analogues with high activity;

— collection of data on quantitative structure–activity relationships (QSAR) for a series of compounds;

— use of bioisosteric transformations.

The synthesis of diverse derivatives based on the purine scaffold provides an evident versatile approach to the development of new synthetic growth regulators, analogues of natural CKs. The adenine moiety can be substituted in several positions (Fig. 4), allowing the synthesis of a wide range of compounds with important biological properties. For example, a large number of derivatives can be synthesized from compounds containing aromatic substituents on the amino group at the 6 position of the ring due to great variety of possible substitutions in the aromatic ring.

Another widely used approach is based on the synthesis of heterocyclic (benzimidazole, pyrimidine, triazine, *etc.*) analogues of purines and non-purine compounds (urea, carbamates and their diverse derivatives), which are generally more synthetically accessible.[153–155] In the past two decades, an extensive library of synthetic compounds based on natural CKs was created. This library was used, for example, to demonstrate that the introduction of substituents at the 1, 3, 7 and 9 positions [at the N(1), N(3), N(7) and N(9) atoms, respectively] of the purine ring (Fig. 4) leads to a significant decrease or even complete loss of

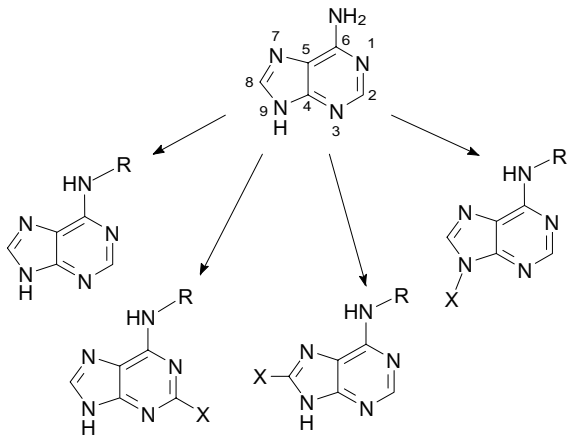


Figure 4. Modifications of adenine for the synthesis of cytokinin-like compounds.

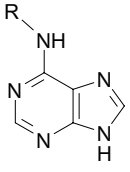
cytokinin activity of the resulting compounds compared to the starting ones.[10] In the majority of recent studies, synthetic CK-like compounds were synthesized by the substitution in the amino group at the 6 position of the purine ring. These adenine derivatives are the first included in the consideration of main groups of synthetic CKs.

5.1. N^6-Substituted adenine derivatives

Investigations of the relationship between the structure of the substituent at the N(6) position and the activity of the purine derivative or its nucleoside demonstrated that a side-chain *trans*-double bond is required for cytokinin activity of compounds that contain substituents composed of 4–7 carbon atoms at the 6 position of the purine ring.[156–158] The introduction of small substituents into the isopentenyl moiety of the compound *tZ* (**1**) has no effect on cytokinin activity of the resulting derivatives. For example, 1′-methylzeatin exhibits strong cytokinin activity in tobacco callus tissues at a concentration of 0.04–1 μmol L^{-1}. The activity of the latter compound is independent of the stereoisomeric composition.[158]

N^6-Aromatic adenines are similar in biological activity to cytokinins **1**, **3** and **6**.[10] For example, BA (**5**), phenyladenine (**20**) and *meta*-topolin (**22**) exhibit activity comparable to that of CKs **1** and **3** and are utilized in biotechnology.[58, 159] Phenyladenine (**20**) displays dual activity, being a ligand for cytokinin receptors and a competitive inhibitor of CKX. Hormonal activity of phenyladenine (**20**) is manifested over a wide concentration range even in plants insensitive to other cytokinin-like growth regulators.[159]

Structures 20–22



20–22

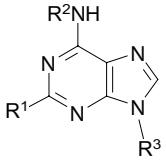
R = Ph (**20**, phenyladenine), $CH_2C_6H_4OMe$-3 (**21**), $CH_2C_6H_4OH$-3 (**22**)

The introduction of small substituents, such as halogen atoms or methyl and OH groups, into the benzene ring leads to a change in cytokinin activity of the compound, but not to its complete loss. The presence of a substituent in *meta* or *ortho* positions increases the activity of the compound, or its activity remains similar to that of cytokinin **5** (BA). By contrast, the introduction of a substituent in the *para* position significantly decreases the activity. The presence of two substituents on the benzene ring generally causes a decrease in cytokinin activity. In the presence of halogen atoms, the activity reduces only slightly and depends on the mutual arrangement of substituents. However, the introduction of bulkier substituents leads to the almost complete loss of activity.[112, 160, 161] Derivatives **5** are characterized by the correlation between cytokinin activity and the ability to inhibit the growth of *E.coli* cells.[156, 157]

Aromatic CKs have higher *in vivo* stability compared to isoprenoid CKs and, consequently, exhibit more pronounced long-term activity.[162] Among CK analogues bearing various substituents at the exocyclic N atom, hydroxy- and methoxy-substituted benzylaminopurines are of particular interest for practical applications. Synthetic CKs containing the substituted benzyl ring, *e.g.*, 6-(3-methoxybenzylamino)- and 6-(3-hydroxybenzylamino)purines (**21**, **22**), are generics of the naturally occurring cytokinins topolins, which were found previously in poplar,[163] and are widely used in biotechnology.[10]

Structural analogues of BA, 6-(2-hydroxy-3-methylbenzylamino)purine (PI-55) (**10**) and N^6-(benzyloxymethyl)adenosine (BOMA) (**12**), are CK antagonists and inhibit the interaction of CKs with CRE1/AHK4 receptors.[60, 62] Another derivative, 6-(2,5-dihydroxybenzylamino)purine (LGR-991) (**11**), acts as an *in vitro* and *in vivo* inhibitor of the CRE1/AHK4 receptor with the same potency as PI-55 but, unlike the latter compound, LGR-991 blocks also the AHK3 receptor and does not exhibit CK-like activity.[61] The high-throughput screening of BA derivatives using the complete set of *Arabidopsis* cytokinin receptors (AHK2–4) revealed highly active compounds, which selectively target individual receptors.[58] Examples are 2-chloro-6-(1-phenylethyl)aminopurine (**23**), which selectively binds to the AHK3 receptor, and 6-benzoylaminopurine (**24**), preferring the AHK2 receptor.

Structures 23–25, 27



23–25, 27

R^3 = H:
R^1 = Cl, R^2 = CH(Me)Ph (**23**);
R^1 = H, R^2 = C(O)Ph (**24**);
R^1 = Cl, R^2 = C_6H_4OMe-3 (**25**, INCYDE);
R^1 = NH–CH(Me)(OH), R^2 = Bn, R^3 = Pr^i (**27**, roscovitine)

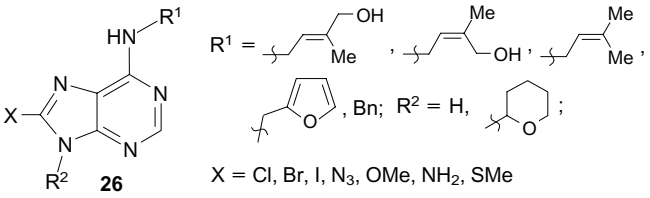
New CK derivatives were synthesized by the introduction of substituents not only into the benzyl ring but into the purine moiety, particularly at the C(2), C(8) and N(9) atoms. These substituted compounds are often referred to as the second-generation CKs. They have properties of CKs but generally do not occur in nature.[10]

The presence of halogen atoms, particularly F or Cl, at the 2 position of the adenine base generally enhances activity.[58, 161, 164] C(2)-Halogen-substituted phenyl- and benzyladenines are potent inhibitors of CKX.[10] Compared to the synthetic CK thidiazuron (**9**) capable of strongly activating CK receptors and inhibiting CKX, most of these compounds are more potent inhibitors of CKX, but have much weaker affinity for the receptors.[165] One of these compounds, 2-chloro-6-(3-methoxyphenyl)aminopurine

(**25**, INCYDE), is used as an inhibitor of CK degradation.[10, 166]

C(8)-Substituted isoprenoid CKs and BA (**26**) exhibit CK activity at higher (by 1–2 orders of magnitude) concentrations, although there are exceptions. For instance, 8-methoxy-Kin, 8-chloro-*t*Z and particularly 8-bromo-*t*Z showed similar or even higher activity compared to unsubstituted CKs. A change in the activity compared to unsubstituted CKs is due both to the structure of CKs and the properties of the substituent at the 8 position of the purine ring.[10, 164]

Structures 26



26

Studies performed in the 1960–1970s reported that derivatives of aromatic CKs containing substituents at the 8 position of the heterocycle, such as C(8)-azidobenzyladenine and C(8)-methylkinetin, exhibit cytokinin activity. Isoprenoid and aromatic derivatives of adenine containing short alkylamino side chains at the C(8) position also have cytokinin activity.[164] Among these compounds, C(8)-halogenated derivatives CKs were found to be the most active although this activity is quite low. The replacement of halogens by bulkier substituents leads to the complete loss of activity.[10, 164]

Cytokinin ribosides containing a sugar moiety attached to the 9 position of the adenine heterocycle (**7**, **8**, **16**, **17**) do not have cytokinin activity and apparently serve as the transport form of CKs.[7, 8, 14] However, ribosides and some other N(9)-substituted CKs displayed cytokinin[167] and sometimes even anticytokinin[168] activity in a number of bioassays. Compounds containing N(9)-substituents (particularly bulky substituents) are not recognized by CK receptors. Evidently, cytokinin activity of these compounds in different bioassays is attributed to their *in planta* activation by enzymatic cleavage of the N(9) substituent (ribose). The presence of a substituent at the N(9) atom can enhance resistance of the compounds to CKX.[167] For example, tetrahydropyranyl and tetrahydrofuranyl derivatives of *meta*-methoxytopolin (**21**) and *meta*-topolin (**22**) are much more active than unsubstituted topolin[162] probably due to their resistance to CKX during the uptake by the cell up to the incorporation into ER, where they presumably lose the substituent at the N(9) atom and, in the form of bases, affect the receptor sensory domain.

It is also interesting that the compound roscovitine, (6-benzylamino-2-(R)-[1-(hydroxymethyl)propylamino]-9-isopropylpurine (**27**) acts as a cyclin-dependent kinase inhibitor and a potent inhibitor of *N*-glycosylation of endogenous CKs, due to which its use in combination with growth regulators significantly increases the efficiency of the latter.[162, 169]

Compounds containing substituents at the N(9) atom and some other positions can show higher transport rate in plant vessels. This has a beneficial effect on the survival of plants without inhibition of root growth, which is generally observed on treatment with typical CKs.[170]

Generally, methods for the synthesis of adenine derivatives involve the standard introduction of substituents into the presynthesized heterocycle *via* alkylation. However, in some hard cases, for example, in the synthesis of isomers of zeatin, special procedures were developed for the synthesis of unsaturated precursors. These approaches are considered in detail in Section 6 of the review.

5.2. Cytokinin-like compounds based on non-purine heterocycles

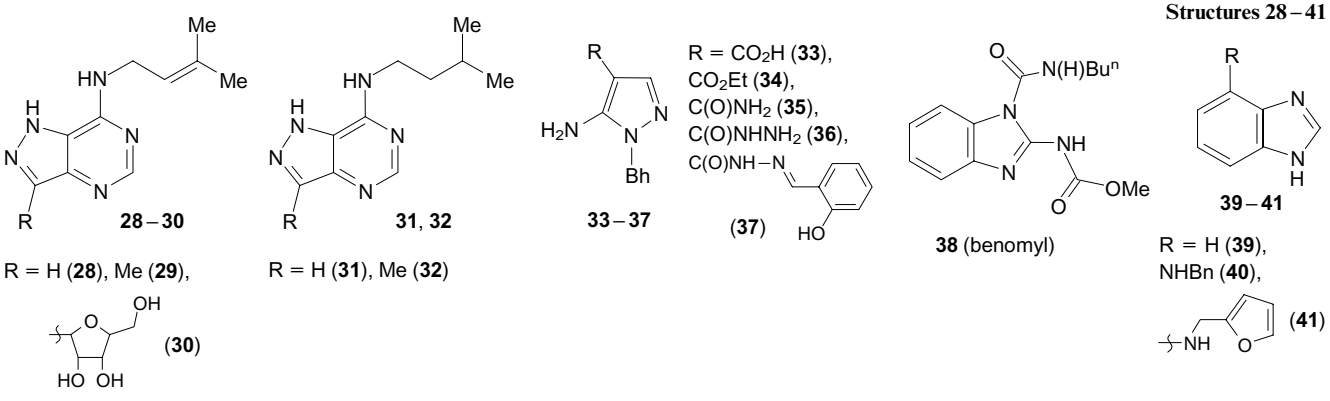
The replacement of the purine core by other heterocycles allows the preparation of compounds with cytokinin-like activity and their antagonists. A series of derivatives and analogues of natural CKs containing a methyl substituent at the N(9) position of the purine ring and(or) containing the pyrazole ring instead of the imidazole moiety have been characterized.[171] Cytokinin activity of iP analogues containing the unsubstituted pyrazole ring (compound **28**) or those bearing the methyl group (**29**) and also of the unsubstituted pyrazolopyrimidine analogue of dihydroisopentenyladenine (**31**) was revealed in a number of conventional assay systems.

Depending on the nature of the substituent at the exocyclic nitrogen atom, certain bioassays of 7-aminopyrazolo[4,3-*d*]pyrimidines revealed both weak cytokinin activity and potent anticytokinin activity, which is most typical of pyrazolopyrimidine derivatives containing saturated hydrocarbon chains (**31**, **32**).[172]

Aminopyrazole derivatives (**33**–**37**) also exhibit cytokinin-like activity, which was confirmed by the leaf disc assay for retardation of chlorophyll degradation and the *Amaranthus* betacyanin bioassay.[173]

A series of compounds with cytokinin-like or anticytokinin activity were found to have fungicidal properties. The fungicide benomyl (**38**) is structurally similar to adenine-

Structures 28–41



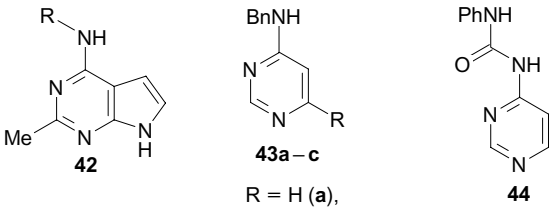
type CKs. It is able to stimulate the soybean leaf growth, but is less potent than Kin.[174]

Benzimidazole (**39**), 4-benzylamino- (**40**) and 4-furfurylaminobenzimidazole (**41**) (synthetic compounds containing the imidazole ring of adenine-type CKs) exhibit cytokinin-like activity at a concentration of 20–40 μmol L^{-1} in the tobacco callus growth-promoting assay and the leaf chlorophyll retention bioassay. The CK activity of two series of 4,5-disubstituted imidazoles was evaluated using the wheat leaf chlorophyll retention bioassay.[175] However, it should be noted that the latter bioassay has low specificity and cannot be considered as a relevant argument in favour of the presence of own cytokinin activity.

Depending on the nature of the substituent at the exocyclic nitrogen atom, this bioassay of substituted pyrrolopyrimidines revealed either potent cytokinin-like activity in the concentration range of 10^{-6}–10^{-5} mol L^{-1} or the anticytokinin activity, by inhibiting Kin in the range $IC_{50} = 6\times10^{-8}$–4×10^{-5} mol L^{-1}. Among these compounds, alicyclic 4-substituted 2-methylpyrrolo[2,3-*d*]pyrimidines (**42**) exhibited the highest anticytokinin activity.[176]

The activity of adenine-type CKs containing the pyrimidine ring was evaluated. 4-Benzylaminopyrimidine **43a** is inactive; however, other pyrimidine derivatives were found to have cytokinin-like activity. Thus, 4-benzylamino-6-methylpyrimidine **43b** exhibited activity similar to that of BA (**5**, 0.01 μmol L^{-1}) but at a 100-fold concentration, 4-(3′-phenylureido)pyrimidine (**44**) showed cytokinin activity in the tobacco callus bioassay at 4.7 μmol L^{-1}, while the 6-chloro derivative (**43c**) displayed more potent cytokinin-like activity.[175]

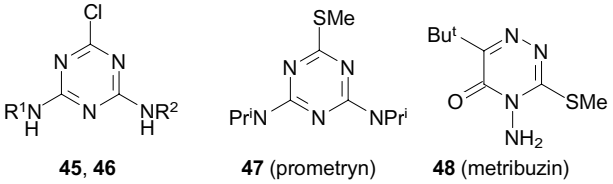
Structures 42–44



Biological properties of such symmetrical triazine herbicides as atrazine (**45**), simazine (**46**) and prometryn (**47**) include cytokinin-like activity. These herbicides improve the callus yield and retard chlorophyll degradation at the optimal concentration lower than 10^{-7} mol L^{-1} (Ref. 175).

Asymmetrical triazine herbicides [metribuzin (**48**)] also exhibit cytokinin-like activity. Metribuzin and BA exerted similar effects on the structural and functional characteristics of the photosynthetic apparatus of agricultural plants (wheat, sugar beet).[175]

Structures 45–48

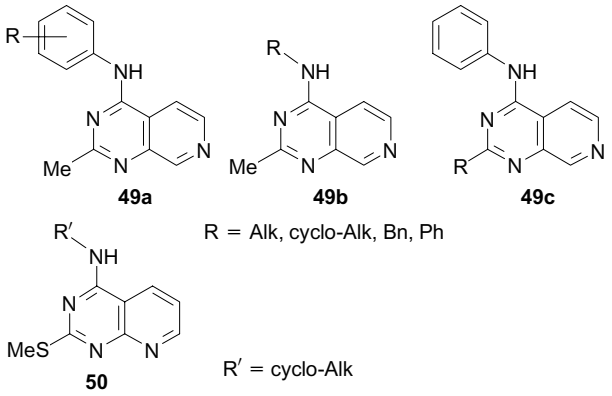


R^2 = Et: R^1 = Pr^i (**45**, atrazine);
R^1 = R^2 = Et (**46**, simazine)

Aniline- and alkylamino-substituted derivatives of 2-substituted pyrido[3,4-*d*]pyrimidines **49a–c** exhibit potent cytokinin-like activity.[177] For example, the activity of *meta*-fluoroaniline-substituted pyridopyrimidine evaluated in the tobacco callus bioassay is similar to that of BA. The introduction of alkyl moieties in the *ortho* position of the aromatic substituent leads to an increase in plant growth regulatory activity, and the replacement of an alkyl or phenyl substituent at the exocyclic nitrogen atom by the benzyl group causes the loss of activity.[177]

4-Substituted 2-(methylthio)pyrido[2,3-*d*]pyrimidines **50** also showed cytokinin-like properties in the tobacco callus bioassay.[178] Their activity directly depends on the nature of the substituent at the 4 position. 4-Alkylamino-substituted pyridopyrimidines are anticytokinins, but they do not stimulate the root system growth. The cytokinin or (indirect) anticytokinin activity of 4-aniline-substituted 2-(methylthio)pyrido[2,3-*d*]pyrimidines directly depends on the position and nature of the substituent in the aniline moiety.

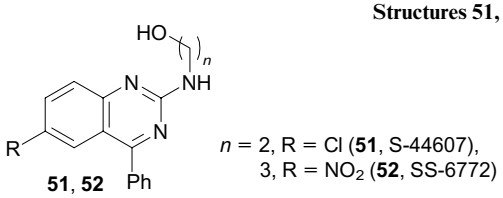
Structures 49, 50



The large-scale screening for the ability of compounds to bind to CK receptors and for root growth promoting activity has been performed.[63] In the series of compounds under investigation, quinazoline derivatives **51** and **52** were found to most efficiently promote the root growth, and compound **13** (S-4893) containing the 4-phenylquinazoline moiety exhibited the highest activity. As mentioned above, S-4893 is a non-competitive inhibitor of the cytokinin receptor CRE1/AHK4. It promotes the root growth in *Arabidopsis* and rice and can be considered as a lead compound for the development of root growth promoters, improving the root system architecture in agronomically important crops.

Aromatic ureas comprise another important class of plant growth regulators with cytokinin or cytokinin-like activity. Examples are *N*,*N*′-diphenylurea (**53**, DPU) and its diverse derivatives: *N*-phenyl-*N*′-1,2,3-thiadiazol-5-ylurea

Structures 51, 52



(**9**, TD) and *N′*-(2-chloro-4-pyridyl)-*N*-phenylurea [**14**, forchlorfenuron, CPPU (4PU-30)].[179,180]

Cytokinin-like phenylureas are similar in biological properties to natural adenine-type CKs. They bind to the same site of cytokinin receptors as do natural N(6)-substituted adenine derivatives (see Fig. 2), resulting in activation of the above-considered two-component signal system (see Fig. 3).[159] They are stable, resistant to oxidases and enhance peroxidase and superoxide dismutase activity. It should be noted that substituted aromatic ureas are generally more efficient than purine-type CKs, due to which they can be used in agriculture and biotechnology for the *in vitro* culturing of cells and tissues.[179] These compounds stimulate the growth of tobacco, pea, ginseng and clover callus and the shoot formation in tobacco, azalea, petunia, mulberry, *etc*. Many urea derivatives also inhibit senescence of individual older leaves or intact plants. They can exert selective effects in certain plants. As an example, let us mention thidiazuron (TD) (**9**) acting as a defoliant for the plant *Malvaceae* and exerting other diverse physiological effects (stimulates the shoot growth in dormant apple trees, lettuce seed germination, sprouting of potato, growth of pumpkin cotyledons and formation of branched trichomes and stomata on floral organs).[180] Thidiazuron (**9**), proven to be genuine CK,[181] is widely utilized for the induction of callus and somatic embryogenesis in biotechnology.[84,175]

The thidiazuron derivatives 3FMTDZ (**54**) and HEDTZ (**55**) do not have cytokinin activity and, unlike TD, do not interact with receptors, but are potent inhibitors of different CKX isoforms, increasing the endogenous CK level.[182]

The synthesis and evaluation of cytokinin activity of a series of phenylurea derivatives made it possible to reveal the structure–activity relationship and formulate the following principles:[180]

— urea derivatives are more active than thiourea derivatives;

— the replacement of one or two protons at the nitrogen atoms in the urea moiety leads to the loss of cytokinin activity;

— the substitution in the aromatic ring enhances activity, compounds bearing *meta*-substituents being more active than those with *ortho*-substituents; compounds with electron-withdrawing substituents are generally more active than those with electron-donating substituents.

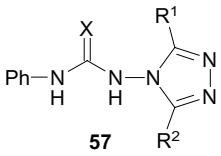
— the substitution in one aromatic ring is more favourable than the simultaneous substitutions in two rings; the pyridine ring is preferable as an aromatic moiety; nonplanar structures cause the loss of activity.

Actually, the activity of 3-(2-chloro-4-pyridyl)-1-phenylurea (**14**) [4PU-30 (CPPU), 10^{-9} mol L^{-1}] evaluated in bioassays is higher than that of BA (**5**), 4PU and DPU (**53**) by approximately 10, 10^2 and 10^4 times, respectively. The thiourea analogue 4PU-30 also exhibits high cytokinin activity (**56**, 4PTU-30, 10^{-9} mol L^{-1}).[175]

More recently, it was found that the dichloro-substituted derivative of 4PU, 3-(2,6-dichloro-4-pyridyl)-1-phenylurea, is among the biologically most active chemical compounds (active at concentrations lower than 10^{-13} mol L^{-1}). 1-Phenylurea derivatives containing a heterocycle at the 3 position were characterized. Thidiazuron (**9**), or 1-phenyl-3-(1,2,3-thiadiazol-5-yl)urea, is registered as a defoliant, was found to be a more potent CK than *tZ* (**1**) in a number of assay systems and exhibits almost the same activity as 4PU-30 (**14**).[175]

1,2,4-Triazole derivatives, which are known to have a wide spectrum of biological activity,[183] were also introduced into ureas. For instance, 1-phenyl-3-(1,2,4-triazolyl)ureas and thioureas **57**,[175,184] and also their salts[185,186] were evaluated as cytokinins and agents increasing the grape, wheat and soybean yield and enhancing the longevity of cut flowers.

Structures 57



57 X = O, S; R^1, R^2 = H, Alk

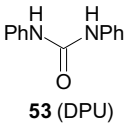
Currently, the synthesis of these compounds is significantly simplified and is performed using the reaction of 1,3-diphenylurea with (4*H*-1,2,4-triazol-4-yl)amine at high temperatures *in vacuo* using distillation of aniline under solvent-free conditions and without toxic reagents.[186]

By varying the structure of the starting ureas, one can synthesize selective growth regulators, which selectively affect plant cells and do not trigger all effects typical of natural CKs. For example, symmetrically substituted diphenylurea derivatives accelerate the growth of the plant root system, without exhibiting cytokinin-like activity associated with the shoot growth, tissue differentiation, *etc*.[180] The *meta*-substitution in the aromatic ring affords diarylureas, which stimulate cell division, while *para*-substituted compounds are effective in senescence retardation and the release of buds from dormancy.

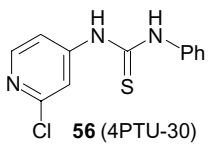
1-Benzyl-3-(4-chlorophenyl)urea (**58**) also induces the root system growth, exhibiting anticytokinin activity.[63]

Hence, in continuation of the search for new cytokinin-like agents among ureas, it was reasonable to synthesize substituted bis-ureas. For instance, the agent EDU (**59**) was synthesized in the study.[187] This compound with cytokinin-like activity effectively protects agricultural crops against ozone injury and enhances phytotoxicity of many herbi-

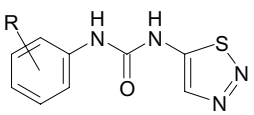
Structures 53–56



53 (DPU)

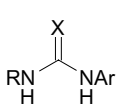


56 (4PTU-30)



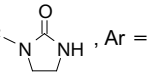
54, **55** R = 3-OCF_3 (**54**, 3-FMTDZ), 2-$(CH_2)_2OH$ (**55**, HEDTZ)

Structures 58–61

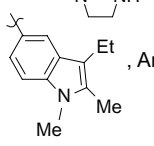


58–61

X = O: R = Bn, Ar = C_6H_4Cl-4 (**58**);

R = 

, Ar = Ph (**59**, EDU);

R = 

, Ar = 3-Py (**60**);

X = S, R = $(CH_2)_2$Py-2, Ar = 2-PyBr-5 (**61**)

cides, which are degraded in plants in the presence of oxygenases.

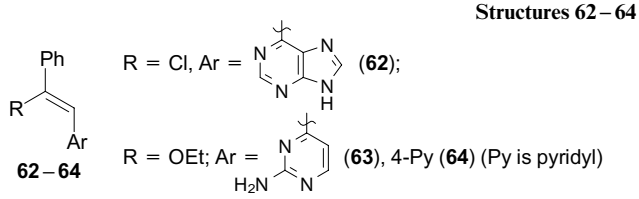
Some EDU analogues were evaluated for the protecting effect against oxygen-derived free radical damage in mammals *via* the induction of endogenous antioxidant enzymes. Among them, the most active compound was a *para*-chlorophenyl derivative, which increases SOD (superoxide dismutase) and CAT (catalase) activity (by 51% and 95%, respectively) in human cells.[175]

Some representatives of 1,3-diheterocyclylureas have important biological properties. 1-Indolyl-3-pyridylureas reduce migraine severity, anorexia and Alzheimer's disease pathology. Among them, the most active compound is 1-(3-ethyl-1,2-dimethylindol-5-yl)-3-(3-pyridyl)urea (**60**). 1,3-Dipyridylthioureas inhibit the replication of human immunodeficiency virus (HIV) and other related viruses, 3-(5-bromo-2-pyridyl)-1-[2-(2-pyridyl)ethyl]thiourea (**61**) being the most active among them.[175]

5.3. Other classes of compounds with cytokinin-like activity

All the above-considered classes of cytokinin-like compounds are characterized by the presence of an aromatic (hetero)cyclic moiety bearing a substituted amino group. The presence of this structural unit is not necessary for growth regulatory activity, and the nitrogen atom of N^6-substituted adenine and its pyrimidine and pyridine analogues can be replaced by the sp^2-carbon atom without the loss of activity.[187] For instance, β-substituted styrylpurines, -pyrimidines and -pyridines (**62**, **63** and **64**, respectively) exhibit cytokinin activity in the tobacco callus bioassay and the *Amaranthus* betacyanin bioassay.[188]

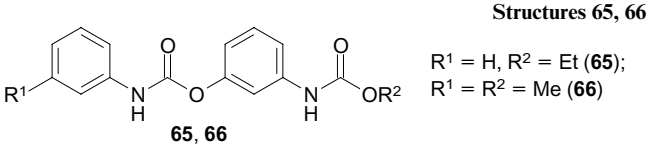
Structures 62–64



62–64

Diverse 2-substituted picolinic acids and pyrimidine-4-carboxylic acids are also effective herbicides and plant growth regulators.[189] A group of compounds with cytokinin activity was discovered upon structural modification of biscarbamates [desmedipham (**65**) and phenmedipham (**66**)] acting as electron transport inhibitors in photosystem II.[190]

Structures 65, 66

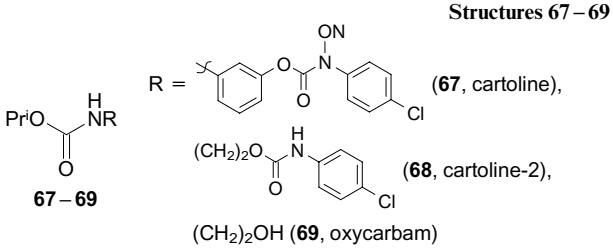


65, 66

In compounds with herbicidal activity, two carbamate functional groups are separated by three carbon atoms of the *meta*-phenylene structural unit. The replacement of these atoms by the ethylene group, with alkyl- and arylcarbamate groups remaining intact, afforded compounds devoid of herbicidal activity but exhibiting cytokinin activity.

Cartoline (**67**) is the first representative in the series of these compounds. It is inexpensive and easy-to-produce cytokinin-like agent with antistress effect. This agent is non-toxic for animals and does not have skin-irritating resorptive-toxic properties. The application of cartoline in spring cereals under field conditions at a dose of 250–500 g ha^{-1} increases the crop yield under drought conditions on the average by 5–9 cwt ha^{-1}. Structural analogues of CKs, known under the common name cartolines, were synthesized in the All-Russian Research Institute of Chemical Means of Plant Protection (VNIIHSZR). Cartolines exhibit antistress activity, mainly protecting plants under water-stress conditions and at critical temperatures. These agents significantly increase drought and frost resistance of plants and have a beneficial effect on the repair and reproductive functions.[191–193] *O*-(4-Chlorophenylcarbamoyl)-2-*N*-(isopropoxycarbonyl)ethanolamine (**68**, cartoline-2)[192–194] and oxycarbam (**69**),[195] which is an intermediate in the synthesis of cartoline-2,[196] are the most effective representatives of this group of compounds.

Structures 67–69



67–69

The synthesis of cartoline-2 (**68**) is accomplished in two steps. The first step involves the acylation of ethanolamine with isopropyl chloroformate giving *N*-2-hydroxyethyl isopropylcarbamate. The latter is transformed into the target product by the reaction with 4-chlorophenyl isocyanate.

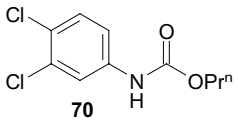
Oxycarbam (**69**) can easily be prepared by the reaction of diisopropyl carbonate, which is synthesized by the reaction of propan-2-ol with triphosgene in the presence of pyridine, with monoethanolamine in the presence of sodium isopropoxide.[195] In some plant species, cartoline-2 and cartoline-4 have the most significant effect on the photosynthesis under stress conditions. Antistress effects of cartolines are higher than those of BA (**5**), Kin (**6**), TD (**9**) or metribuzin (**48**).[192, 193]

Non-purine CKs have a narrower range of physiological activity. For example, cartolines do not regulate chloroplast differentiation, do not induce the production of nitroreductase and have no specific effect on the stem and hypocotyl growth and on the promoted cell growth in etiolated leaves or isolated cotyledons. The protective effect typical of CKs was exhibited by cartolines at a dose as low as 0.04 mg in plants at high and low temperatures, under drought stress and other detrimental environmental conditions.[192, 195]

More recently, the cartoline-2 analogues, *N*-aryl-*N*′-alkoxycarbonylaminoethylureas, and their oxalyl derivatives, *N*-aryl-*N*′-alkoxyoxalylaminoethylureas, were synthesized by a similar procedure, and these compounds were shown to exhibit not only growth regulatory activity but also interesting and more potent antidote (antiherbicidal) and antistress activity.[197–200] Hence, these compounds can be considered as candidates for the use in agriculture.

Some substituted carbamates exhibit anticytokinin activity. An example is propyl *N*-(3,4-dichlorophenyl)carbamate (**70**).[63]

Structure 70



70

In recent years, other classes of organic compounds were found to have plant growth stimulating activity. In particular, such rare agents as sidnonimines,[201] which belong to mesoionic heterocyclic compounds and have diverse physiological activity, act as plant growth stimulators.[202] Sidnonimines extend the range of effective agents with plant growth stimulating activity at superlow doses (1–10 g of compound per ton of seeds). The method for the synthesis of these compounds involves the intramolecular cyclization of nitroso derivatives of aminonitriles and subsequent acylation of the intermediates with carboxylic or sulfonic acid anhydrides.[203, 204]

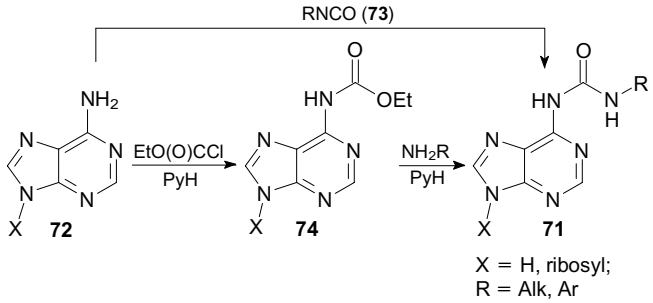
6. Methods for the synthesis of cytokinin-like compounds

Synthetic CKs belong to diverse classes of compounds, including both BA derivatives and phenylureas (non-purine compounds). Apart from cytokinin activity, phenylureas have plant growth regulatory, antimutagenic and pesticide properties and exhibit activity against phytoviruses.[174, 175, 187] Synthetic growth regulators can be useful in crops due to a number of advantages over natural CKs (see above). The recently developed biologically active compounds with cytokinin-like activity have wide practical applications, which will be most probably extended in future.[175, 198, 200] In most cases, the activity of the above synthetic cytokinin analogues is due to the presence of the urea group in the molecule. The carbamide and carbamate groups play a significant role in a series of target compounds, cartoline-2 analogues. The synthetic routes to compounds containing these moieties are considered below. Methods for the synthesis of BA derivatives are described in reviews.[9, 10]

6.1. Synthesis of *N*-substituted adenines

Methods for the synthesis of adenine derivatives are generally based on the functionalization of the heterocyclic core, in particular, by alkylation.[156–158] Some 6-ureidopurines (**71**) and their nucleosides[205–207] are synthesized from aminopurine **72** and its ribosides by the reaction with isocyanates (**73**) or the sequential acylation of the exocyclic nitrogen atom with ethyl chloroformate and aminolysis of the resulting carbamate **74** (Scheme 1).

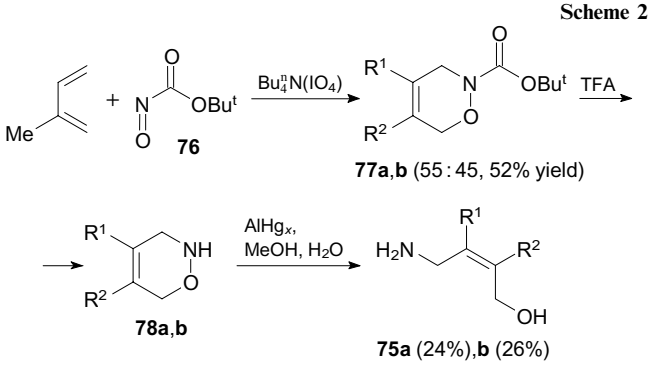
Scheme 1



For some sophisticated syntheses, *e.g.*, for the synthesis of zeatin isomers **1** and **2**, special methods for the preparation of unsaturated precursors were developed. These methods are considered in detail below.

Both isomers of zeatin play a key role in plant development and commonly occur in nature.[8] However, although the chemistry of *trans*-(*E*)-zeatin (**1**) is well known and this compound is readily available, the synthesis of pure *cis*-(*Z*)-zeatin (**2**) remains a challenge because of difficulties with the isolation of pure (*Z*)-4-amino-2-methylbut-2-en-1-ol (**75a**). Several methods developed for the synthesis of this amino alcohol afford an inseparable mixture of isomers, from which the desired compound can be isolated in low yield. The reported five-step synthesis of **75a**[208] gave good outcome but cannot be scaled-up. Hence, Tolman *et al.*[209] modified one of the known methods based on the Diels–Alder reaction for metastable nitrosoformate **76**. The latter compound was prepared by the *in situ* oxidation of *tert*-butyl *N*-hydroxycarbamate and then reacted with isoprene to form a mixture of *tert*-butyl 5- and 4-methyl-3,6-dihydro-2*H*-1,2-oxazine-2-carboxylates **77a**,**b** (Scheme 2). After the removal of ester groups and the reduction of the resulting 3,6-dihydro-2*H*-1,2-oxazines **78a**,**b** to amino alcohols **75a**,**b**, the latter were separated by chromatography into (*E*)-**75a** and (*Z*)-isomers (**75b**). Amino alcohol **75a** was produced as an oxalate by the three-step synthesis in an overall yield of 10%.

Scheme 2

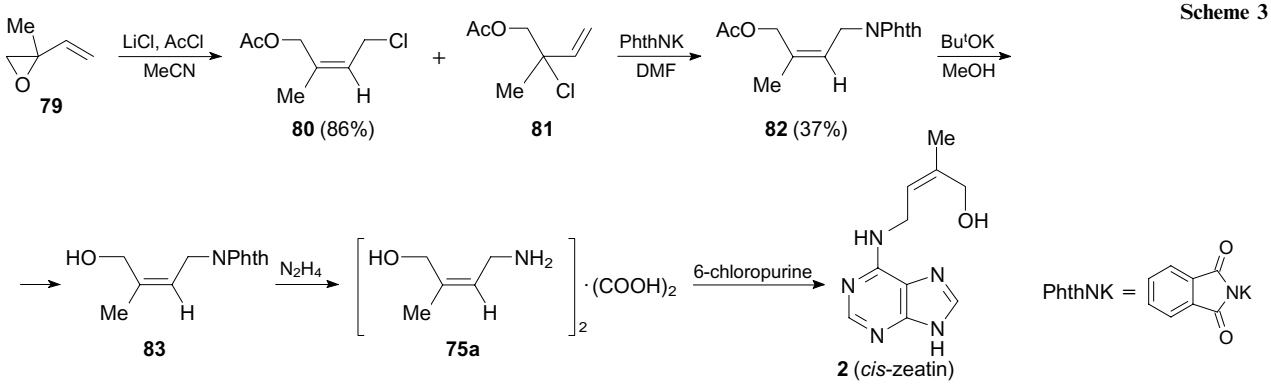


75, **77**, **78**: R^1 = H, R^2 = Me (**a**); R^1 = Me, R^2 = H (**b**)

A new stereoselective method for the synthesis of *Z*-isomer **75a** (Scheme 3) was reported in the recent study.[210]

Commercially available 2-methyl-2-vinyloxirane (**79**) was used as the starting compound. The stereoselective rearrangement of this compound with acetyl chloride in the presence of lithium chloride affords a mixture of (*Z*)-1-acetoxy-3-methylbut-2-enyl chloride (**80**) and a small amount of the isomer (**81**). The reactions of compounds **80** and **81** with potassium phthalimide occur *via* different pathways. By-product **81** gives dehalogenated 1-acetoxy-2-methylbutadiene, which is separated during the wokup of the reaction mixture. By contrast, alkene **80** reacts with PhthNK to form the expected phthalimide **82**. The alcoholysis of the acetoxy group of compound **82** affords hydroxy derivative **83**, which is subjected to hydrazinolysis to give the corresponding amine, (*Z*)-4-hydroxy-3-methylbut-2-enylamine **75a**, a side chain of the cytokinin *cis*-zeatin. Compound **75a** is isolated as oxalate by the precipitation. After four steps, the overall yield of the product was 17%. The reaction of **75a** with 6-chloropurine gave *cis*-zeatin (**2**)

Scheme 3

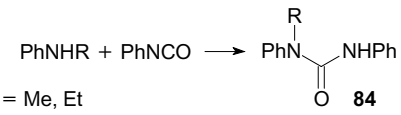


with 99.5% purity. This synthesis comprises a simple efficient procedure without the need for chromatographic separation of isomeric by-products, which facilitates the scaling up of the synthesis of *cis*-zeatin.[210]

6.2. Synthesis of substituted ureas

The reaction of organic isocyanates with amines, *e.g.*, of phenyl isocyanate with aniline derivatives, affords substituted ureas (**84**) (Scheme 4).[211] This is one of the most commonly used approaches to the synthesis of unsymmetrical ureas with cytokinin activity.

Scheme 4

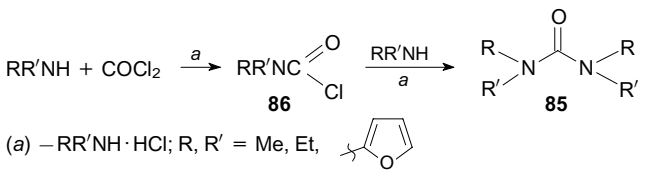


Generally, the synthesis is performed using equimolar amounts of reactants or a small excess of isocyanate (more rarely, an excess of amine). The process is performed in an organic solvent, but in some cases water is used as the solvent, because isocyanates react with water much more slowly than with amines. In most cases, substituted ureas are produced in high yields, often in nearly quantitative yields. Aliphatic amines react much faster than aromatic amines, and the reactivity of isocyanate increases with increasing electrophilicity of the substituent that is present in the molecule.

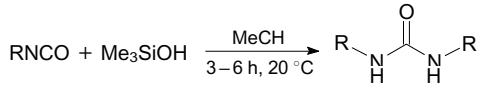
Symmetrical dialkyl-, diaryl- and alkylarylureas (**85**) can be prepared by the phosgenation of the corresponding amines in organic solvents.[211,212] The reaction of amine with phosgene includes two steps. The initially formed carbaminoyl chloride (**86**) also reacts with the starting amine (Scheme 5). Hydrogen chloride released during the reaction binds to the amine to form amine hydrochloride. The rate of the reaction of amines with phosgene is high and increases with increasing basicity of the starting amine.

An efficient one-pot synthesis of symmetrically substituted ureas (**87**) is based on the reaction of isocyanates with

Scheme 5



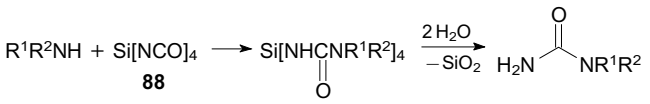
Scheme 6



trimethylsilanol and allows the preparation of target products in high, nearly quantitative, yields (Scheme 6).[213]

Another approach to the synthesis of *N*-mono- and *N*,*N*-disubstituted ureas involves the reaction of silicon tetraisocyanate (**88**) with primary or secondary amines (Scheme 7).[214] The advantage of silicon tetraisocyanate is its solubility in main organic solvents, which are also suitable for amines. The reaction of primary aromatic amines is rapid and is accompanied by an exothermic effect, whereas the reaction of secondary amines requires prolonged heating. The reactivity of amines in this reaction decreases in the following order: $RNH_2 > R_2NH > ArNH_2 gg ArNHR \gg Ar_2NH$ (R is alkyl, aralkyl; Ar is aryl).

Scheme 7



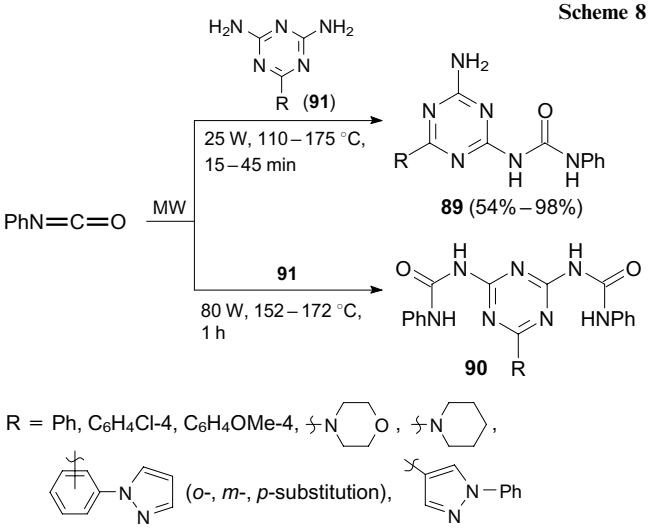
The microwave-assisted synthesis of triazinylmono- (**89**) and -bisureas (**90**) from triazine (**91**) and phenyl isocyanate using minimum amounts of the solvents (toluene or dioxane) enables the control over the reaction temperature and affords monourea (R = Ph) in 68% yield in only 15 min (Scheme 8).[215] The optimal reactant ratio (PhNCO : **91**) was found to be 1 : 2.1; nevertheless, the use of an excess of the isocyanate did not result in the formation of bisureas.

Bisureas **90** are produced under more severe conditions, such as higher irradiation power, higher temperature and longer reaction time.

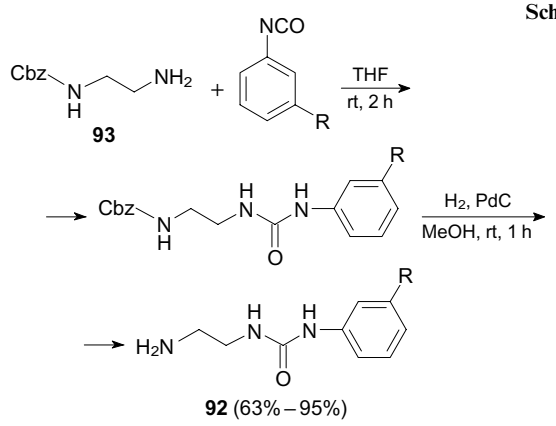
The synthesis of aryl(aminoethyl)ureas (**92**) is based on the reaction of aryl isocyanate with Cbz-protected ethylenediamine (**93**). After the deprotection, the corresponding amino compounds **92** were obtained in high yield (Scheme 9).[216]

Another one-pot method for the synthesis of unsymmetrical ureas (**94**) consists in the Curtius rearrangement and is accomplished under microwave irradiation of com-

Scheme 8



Scheme 9

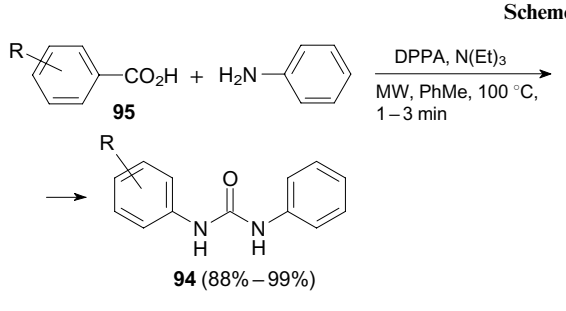


mercially available (hetero)aromatic acids (**95**) and amines in the presence of diphenylphosphoryl azide (DPPA) and triethylamine within a few minutes (Scheme 10).[217]

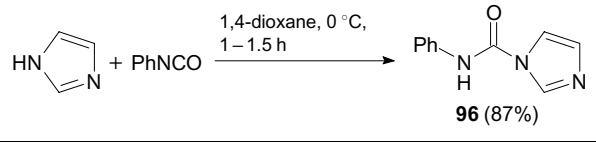
N-Phenyl-*N*′-imidazolylurea (**96**) was synthesized by the reaction of phenyl isocyanate with imidazole in dioxane (Scheme 11).[218] The evaluation of antidepressant activity of this compound in mice demonstrated that it is a potential antidepressant agent.

Recently, a one-pot method was developed for the synthesis of aryl isocyanates from arylamines in a CO_2 atmosphere under mild conditions without using metals.[219]

Scheme 10

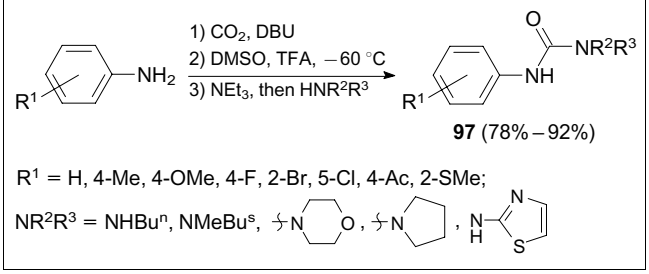


Scheme 11



Intermediate carbamic acid, produced from arylamine and CO_2 in the presence of DBU, is dehydrated with activated sulfonium reagents (generated *in situ* from DMSO and TFA) to give the corresponding isocyanate, which reacts with various amines and alcohols to produce unsymmetrical ureas (**97**) and carbamates, respectively (Scheme 12).

Scheme 12

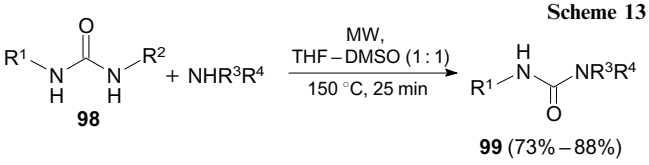


6.3. Transamidation of substituted ureas

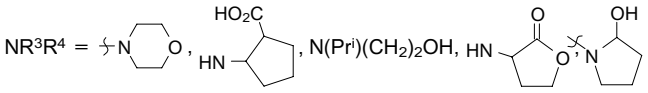
Advances in experimental approaches (microwave irradiation, reactions in an aqueous medium, *etc.*) allow the further development of the transamidation approach for the synthesis of ureas with cytokinin activity.

The microwave-assisted transamidation reaction of cyclopentyl- or isopropyl-substituted urea (**98**) with various amines taken in excess (5–10 equiv.) gave ureas **99** (Scheme 13).[220] The reactions proceed with retention of the absolute configuration of chiral substituents. In the author's opinion, microwave irradiation promotes the cleavage of a weaker C(O)–N bond in the sterically less hindered amine moiety, resulting in the formation of an intermediate isocyanate compound containing a relatively bulky group.

Scheme 13



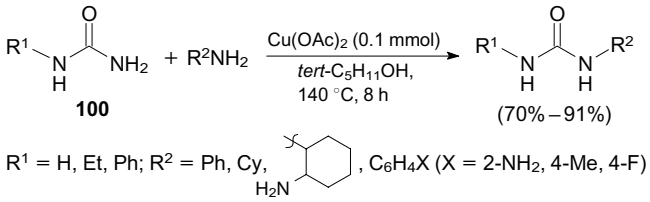
R^1 = CBnAr^{1}Ar^2, CBnArHetAr, *etc.*; R^2 = cyclo-C_5H_4, Pr^i;



The copper acetate-catalyzed reaction of ureas **100** (phenyl- and ethylureas) with aryl- and cycloalkylamines or diamines (Scheme 14) giving the final product in 70%–91% yield has been reported.[221] It was assumed that the transamidation occurs *via* the replacement of the less nucleophilic amine by the more nucleophilic one.

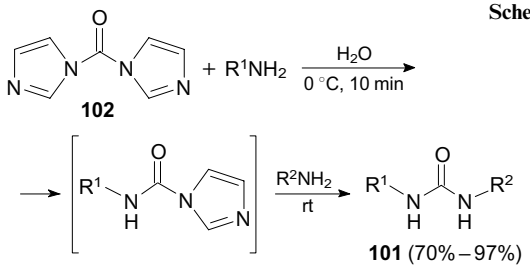
A method for the synthesis of unsymmetrically substituted ureas (**101**) from 1,1′-carbonyldiimidazole (**102**, CDI) and a series of amines in water (Scheme 15) has been

Scheme 14



100

Scheme 15



102

101 (70% – 97%)

R^1, R^2 = $(CH_2)_2OMe$, Bn, $C_6H_3(OMe)_2$-3,4, $C_6H_4(CO_2Et)$-4, 3-Py(OMe)-6, *etc.*

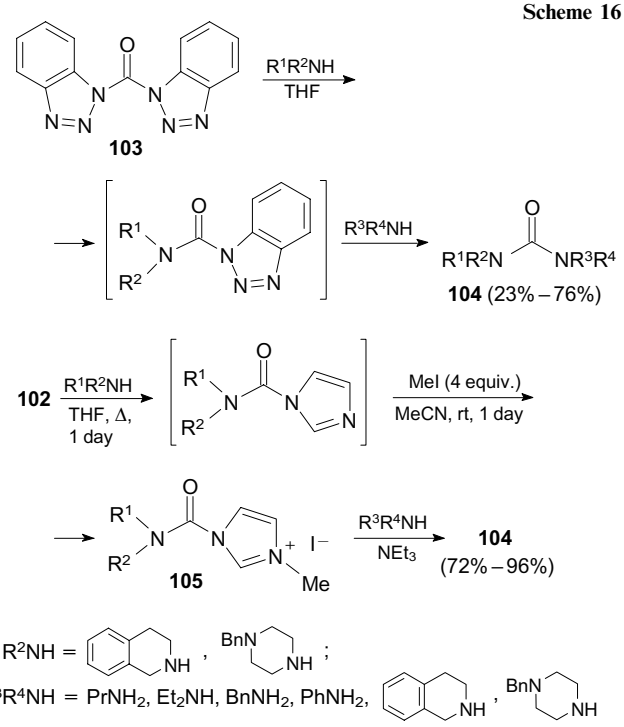
described.[222] After the completion of the reaction with the first amine, the reaction mixture is heated and the second amine is added. A significant advantage of the method is that the synthesis can be performed in water as the solvent.

1,1′-Carbonylbisbenzotriazole (**103**) can be used in a similar way (Scheme 16).[223] 1,1′-Carbonylbisbenzothriazole is a safe, mild and versatile reagent, which was the first alternative to phosgene in the synthesis of unsymmetrical ureas. This compound was utilized to prepare various *N*,*N*,*N*′,*N*′-tetrasubstituted unsymmetrical ureas (**104**). The reactions of cyclic and aliphatic amines can be accomplished at room temperature to prepare the corresponding ureas in 50% – 76% yields, while the reactions of aromatic amines occur only at reflux and give products in lower yields (23% – 40%). However, 1,1′-carbonylbisbenzotriazole (**103**) is not as accessible as CDI for large-scale processes.[223]

More recently, CDI (**102**) was employed for the preparation of tetrasubstituted ureas **104** (see Scheme 16). Since carbamoylimidazoles exhibit low reactivity towards amines, they are activated *via* *N*-alkylation of the imidazole moiety with iodomethane to prepare resonance-stabilized imidazolium salts (**105**), which readily react with secondary amines at room temperature to give ureas **104** in high yields.[223] It should be noted that, as opposed to the method for the synthesis of bisureas in water[222] described above, this method enables the use of secondary amines.

A method was developed for the synthesis of *N*-methylcarbamoylimidazole (**106**) in quantitative yield from CDI and $MeNH_3Cl$ (Scheme 17).[224] Product **106** is a crystalline water-stable compound and serves as an efficient substitute for methyl isocyanate. The reaction of *N*-methylcarbamoylimidazole with nucleophiles, such as amines, protected and unprotected amino acids, thiols and alcohols, occurs in the presence of triethylamine. *N*-Methylureas, carbamates and thiocarbamates are synthesized in high yields in organic solvents or in water. Other primary ammonium hydrochloride salts, in particular protected α-amino acid salts, give the corresponding *N*-alkylcarbamoylimidazoles in high yields and can also be transformed into ureas in high yields without the formation of intermediate isocyanates.

Scheme 16



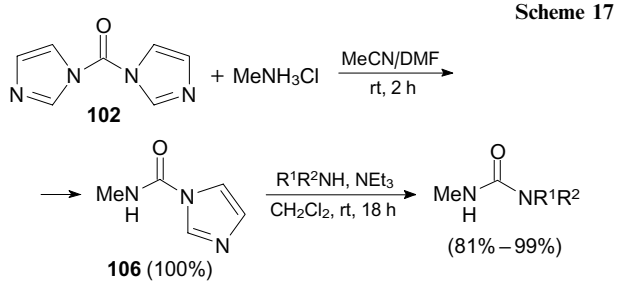
103

105

R^1R^2NH = , ;

R^3R^4NH = $PrNH_2$, Et_2NH, $BnNH_2$, $PhNH_2$, , ,

Scheme 17



102

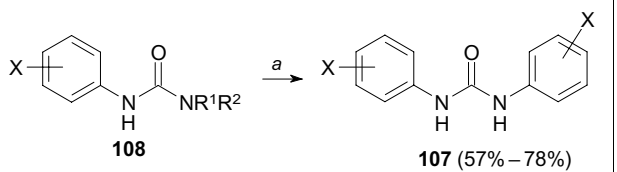
106 (100%)

R^1R^2NH = $NHEt_2$, $NHBn_2$, NH_2Bn, , , $H_2N(CH_2)_2Ph$, $H_2NCH_2C_6H_3(OMe)_2$-2,4, $NH(Me)(CH_2)_2C_6H_3(OMe)$-3,4

A method for the synthesis of symmetrical substituted ureas (**107**) from unsymmetrical *N*-aryl-*N*′,*N*′-dialkylureas and *N*-aryl-*N*′-alkyl(alkylphenyl)ureas (**108**) in the presence of diethylcarbamoyl chloride (Scheme 18) has been reported.[225]

The benzoic acid-catalyzed transamidation of ureas under metal-free conditions (Scheme 19) has been accomplished.[226] The highest yield of product **109** was achieved in

Scheme 18

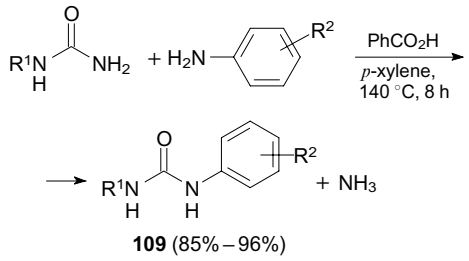


108

107 (57% – 78%)

(*a*) $Et_2NC(O)Cl$, dioxane, 50 – 60 °C, 4 – 12 h;
X = H, 4-Cl, 2,4-Cl_2, 2-NO_2, 3-NO_2, 4-NO_2;
R^1, R^2 = H, Me, Et, Pr^n, Bu^n, $(CH_2)_2Ph$

Scheme 19

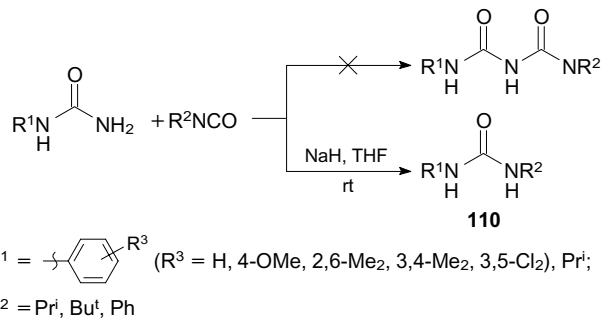


R^1 = H, Et, Ph; R^2 = H, 2-NH_2, 4-Me

the reaction performed under reflux in *p*-xylene, and the reaction required the minimum time.

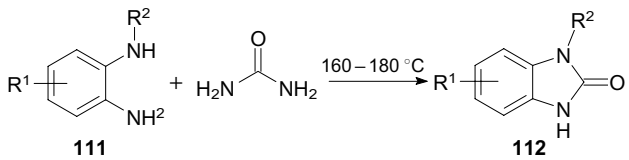
A method was developed for the synthesis of *N*,*N*′-substituted ureas based on the cross-coupling of monosubstituted ureas and commercially available isocyanates in the presence of sodium hydride.[227] Symmetrical and unsymmetrical ureas **110** were synthesized in one step in good yields (58%–91%). This was a surprising result because the reaction was expected to produce a compound containing two carbamide groups (Scheme 20).

Scheme 20



The reactions of ureas with bifunctional compounds giving cyclic products with a carbamide moiety in the ring, oxoimidazolidines, were described in the literature. For example, the reaction of urea with primary and secondary diamines (**111**) on heating above 160 °C, allowing the use of alcohols as solvents, afforded products **112** (Scheme 21) in high yields (up to 86%).[228]

Scheme 21



R^1 = H, Alk, Ar, Hal, OAlk, CO_2H, CN; R^2 = H, $CH_2C_6H_3F$-4

6.4. Synthesis of cartoline analogues

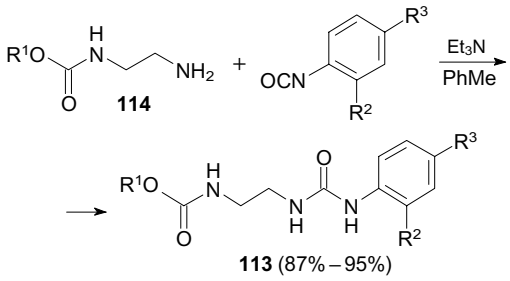
A characteristic structural feature of synthetic cytokinin analogues, such as carbamate and urea derivatives [phenmedipham (**66**), cartoline (**67**) and cartoline-2 (**68**)],[175, 180, 192] is the presence of two toxophoric groups separated by a polymethylene bridge. It would be expected that *N*-(2-imidazolidinon-1-ylethyl)-*N*′-phenylcarbamide (**59**, EDU) containing two urea groups will exhibit cytokinin activity, but this compound was evaluated only for the ability to protect plants against atmospheric ozone.[187]

The compound EDU (**59**) was found to increase the content of superoxide dismutase, catalase and glutathione peroxidase in plant cells. These enzymes are the main antioxidant enzymes that scavenge superoxide radicals, hydrogen peroxide and free radicals in plant and animal cells. It is worth noting that the effect associated with an increase in the concentration of the enzymes, which are involved in the protection of cells against oxidative stress, is observed also in assays in animal cell cultures.[229]

A comparison of the structures of cartoline-2 (**68**) and EDU (**59**)[187, 191, 229] led the authors[200] to the conclusion that compounds containing different functional groups (carbamate and urea), which are responsible for activity and are separated by an ethylene bridge, can exhibit growth regulatory activity, apparently, higher than that of related biscarbamates, because the strength of their binding to the corresponding biological targets will be sufficient for additional types of activity.

The target *N*-aryl-*N*′-alkoxycarbonylaminoethylureas (**113**) were synthesized by the reaction of β-aminoethylcarbamic acid alkyl esters (**114**) with isocyanates (Scheme 22).[198]

Scheme 22

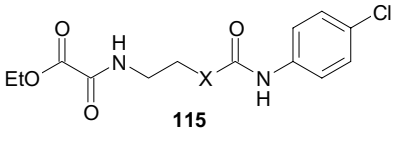


R^1 = Bu^s: R^2 = R^3 = H, Me; R^2 = R^3 = Me;
R^1 = Bu^n, R^2 = Me, R^3 = H;
R^1 = Pr^i: R^2 = R^3 = H, Me; R^2 = H, R^3 = CO_2Et

Many derivatives of oxalic acid, in particular its amides, exhibit biological activity.[230, 231] Hence, oxalic acid derivatives, structural analogues of carbamoylethylureas and cartoline-2 (**68**), were proposed as an alternative to the latter compounds. These derivatives were poorly characterized in terms of biological activity. *N*-Aryl-*N*′-alkyloxamoylethylureas **115** were synthesized in a similar way to the formates.

Biologically active carbamates hold promise due to their synthetic accessibility. These compounds can easily by prepared by the reaction of *N*-β-hydroxyoxamates with appropriate isocyanates in the presence of triethylamine, resulting in the formation of bifunctional compounds bearing oxamate and carbamate functional groups.[198, 199] However, they are scantily soluble in water, which is typical of

Structure 115



X = O, NH

this class of compounds. A possible solution to this problem is to encapsulate such low-molecular-weight physiologically active compounds in water-soluble synthetic and natural polymers.

Thermally sensitive water-soluble *N*-vinylcaprolactam – *N*-vinylimidazole copolymers were prepared as substrates.[232]

Biologically active β-oxalylamino-substituted *O*-ethyl carbamate was encapsulated in micelles of hydrolyzed and non-hydrolyzed casein.[232, 233] The atomic force microscopy study showed that the controlled hydrolysis of casein with trypsin afforded monodisperse nanoparticles with an average diameter of 22 nm and a dispersion of 5 nm. These particles were found to encapsulate larger amounts of chlorine-containing alkyl carbamate compared to the starting casein micelles. The solubilization gave a colloidal form of alkyl carbamate.

The formation of complexes of low-molecular-weight compounds with water-soluble polymers provides a water-soluble colloidal form (a stable micellar solution with a high concentration of the physiologically active component) convenient for practical applications of the compounds in agriculture.

7. Conclusion

Cytokinins play an essential role in the plant growth, development and physiological activities. The biological activity of these phytohormones is mediated *via* the cytokinin signal transduction mechanism. This pathway in plants is carried out *via* an analogue of two-component signal-transduction system comprising sensor histidine kinase protein that transports the high-energy phosphate and a response regulator (transcription factor). Cytokinins bind to the CHASE domain of histidine kinase, which triggers the signal transduction chain. Numerous compounds are known to exhibit cytokinin activity. All these compounds belong to a limited number of chemical classes, such as amino-substituted heterocycles and ureas. Natural cytokinins are N^6-substituted adenine derivatives. A large group of synthetic cytokinins includes mainly purine derivatives and related substances, and also arylureas. Most of the latter molecules are synthetic derivatives; nevertheless, some of them exhibit growth regulatory activity comparable to or even higher than that of natural cytokinins. Among other compounds with cytokinin-like activity, relatively poorly characterized carbamates and their analogues are worth mentioning. These substances can be synthesized by relatively simple methods, and the presence of functional groups in these cytokinin-like molecules makes it possible to significantly expand the range of available derivatives as highly promising compounds with cytokinin activity. Many cytokinins, their derivatives and(or) structural analogues are already used in practice in such important areas as biotechnology, plant growing, medicine and cosmetics and obviously hold promise for future applications.

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