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STUDY OF THE BENZYLATION REACTIONS OF 6-AMINOPURINE AND ITS IMMOBILIZATION PROPERTIES IN HYDROGEL CAPSULES

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Abstract: 6-Aminopurine is an important heterocyclic compound exhibiting pronounced biological activity and serving as a precursor of plant growth regulators and coordination ligands. In this study, the benzylation reactions of 6-aminopurine were investigated under various reaction conditions in order to obtain selectively substituted derivatives. The influence of solvent polarity, base strength, temperature, and reagent ratio on the direction of N-alkylation was analyzed, with particular attention to substitution at the N7 and N9 positions of the purine ring. The synthesized benzylated products were isolated and structurally characterized using FT-IR, UV–Vis and NMR spectroscopy, confirming successful modification of the heterocyclic system.

Furthermore, the obtained derivatives were immobilized into hydrogel capsules based on hydrophilic polymer matrices to evaluate their encapsulation efficiency and release behavior. The hydrogel system demonstrated high loading capacity and gradual diffusion of active molecules into aqueous media, indicating controlled-release properties. The interaction between the heterocyclic molecules and the polymer network was found to be governed by hydrogen bonding and weak coordination interactions, improving stability of the active compound.

The combined results show that benzylated derivatives of 6-aminopurine can be effectively incorporated into hydrogel carriers, forming stable hybrid systems with potential application in agriculture as prolonged-action plant growth regulators and stress-protective agents.

Keywords: 6-aminopurine, benzylation, N-alkylation, purine derivatives, hydrogel capsules, immobilization, controlled release, plant growth regulators.

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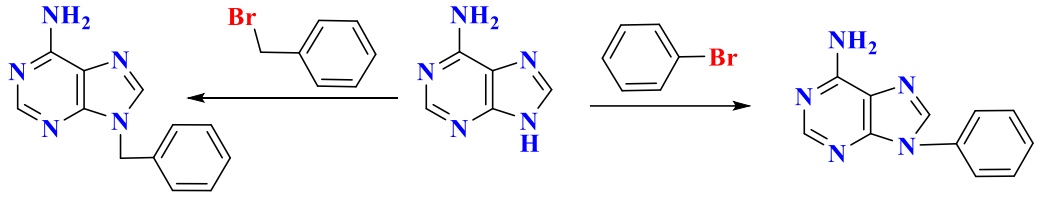
Introduction

Heterocyclic nitrogen-containing compounds occupy a central place in modern organic and bioorganic chemistry due to their wide occurrence in natural biomolecules and their diverse biological functions. Among them, purine derivatives represent one of the most significant classes, forming the structural basis of nucleic acids, coenzymes, alkaloids and plant hormones. 6-Aminopurine (adenine) is especially important because small structural modifications in the purine ring drastically alter its biochemical activity. Substituted adenine derivatives act as enzyme regulators, metal-binding ligands and physiologically active agents in living organisms.



Alkylation reactions of purines are a key method for modifying their physicochemical and biological properties. The purine ring contains several nucleophilic nitrogen atoms (N7 and N9) capable of electrophilic substitution, which leads to the formation of regioisomeric products. Among various alkyl groups, the benzyl fragment is of particular interest because it increases lipophilicity, membrane permeability and biological stability of the molecule. However, selective benzylation of 6-aminopurine remains a complex problem due to competing N7- and N9-substitution pathways. The reaction direction strongly depends on solvent polarity, base strength, temperature and the nature of the alkylating agent. Therefore, studying the factors controlling regioselectivity is necessary for obtaining target biologically active derivatives.

Recently, purine derivatives have also attracted attention as functional components of hybrid polymer materials. Immobilization of low-molecular-weight bioactive compounds into hydrogel matrices allows stabilization of labile molecules, reduction of toxicity and achievement of prolonged release. Hydrogels are three-dimensional cross-linked polymer networks capable of absorbing large amounts of water while retaining structural integrity. Due to their biocompatibility, high sorption capacity and diffusion-controlled release behavior, hydrogel capsules are widely used as carriers for agrochemicals, pharmaceuticals and growth regulators.

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Incorporation of benzylated adenine derivatives into hydrogel capsules may create controlled-delivery systems with improved efficiency and environmental safety. The purine ring contains donor atoms able to form hydrogen bonds and weak coordination interactions with polymer functional groups, which enhances retention of the active compound inside the matrix and regulates diffusion into the surrounding medium.

The present work is devoted to the investigation of benzylation reactions of 6-aminopurine under different conditions, identification of substitution patterns, and evaluation of immobilization properties of the obtained derivatives in hydrogel capsules. Establishing the relationship between molecular structure and release behavior is important for developing prolonged-action biologically active materials applicable in agricultural biotechnology and environmentally safe plant growth regulation.

Materials and Methods

6-Aminopurine (adenine, analytical grade) was used as the initial heterocyclic compound. Benzyl chloride and benzyl bromide served as benzylating agents, while potassium carbonate, sodium hydride and triethylamine were used as bases for activation of the purine nitrogen atoms. Dimethylformamide (DMF), dimethyl sulfoxide (DMSO) and ethanol were applied as solvents. All reagents were of reagent grade and used without additional purification. Distilled water was used throughout the immobilization and hydrogel preparation procedures. For hydrogel synthesis, acrylic acid and acrylamide were chosen as hydrophilic monomers, N,N′-methylenebisacrylamide as crosslinking agent, ammonium persulfate as radical initiator and N,N,N′,N′-tetramethylethylenediamine as accelerator.

The benzylation reaction was carried out in a dry round-bottom flask equipped with magnetic stirring. 6-Aminopurine was suspended in anhydrous DMF and treated with potassium carbonate in order to generate the purine anion. After activation, benzyl chloride was added dropwise under continuous stirring and the mixture was heated at 70–80 °C for several hours under reflux. The progress of the reaction was monitored by thin layer chromatography. Upon completion, the mixture was cooled and poured into cold water, leading to precipitation of the benzylated derivative. The product was filtered, washed with water and recrystallized from ethanol. Parallel reactions were conducted in DMSO and ethanol and also in the presence of sodium hydride as a stronger base to study

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the influence of reaction conditions on regioselectivity of substitution at the N7 and N9 positions of the purine ring.

The synthesized compounds were characterized using physicochemical and spectral methods. FT-IR spectroscopy in the range 4000–400 cm^{-1} was applied to identify functional groups and confirm N-alkylation, UV–Vis spectroscopy was used to observe electronic transitions in the purine chromophore, and ^1H NMR spectroscopy was performed to determine substitution position and aromatic proton environment. Melting point determination was additionally used as a purity control.

Hydrogel capsules were prepared by free-radical polymerization in aqueous medium. Acrylic acid and acrylamide were dissolved in distilled water in equimolar ratio, followed by addition of N,N′-methylenebisacrylamide as crosslinking agent. Ammonium persulfate and tetramethylethylenediamine were introduced to initiate polymerization and the solution was kept at moderate temperature until formation of a stable three-dimensional gel network. The formed hydrogel was thoroughly washed with water to remove residual monomers and dried to constant mass.

Immobilization of benzylated 6-aminopurine derivatives was carried out by swelling-diffusion method. Dry hydrogel samples were immersed in aqueous solutions of the synthesized compounds and kept at room temperature for 24 hours. During swelling, active molecules penetrated into the polymer matrix and were retained by hydrogen bonding and weak donor–acceptor interactions with functional groups of the hydrogel network. Encapsulation efficiency was determined spectrophotometrically from the decrease of concentration in the external solution.

Release kinetics was studied by placing the loaded hydrogel capsules into distilled water at constant temperature. At defined time intervals aliquots were taken and analyzed by UV spectroscopy at the absorption maximum of the compound. The cumulative release percentage was calculated to evaluate the diffusion-controlled behavior of the system. All measurements were performed in triplicate and the averaged values were reported.

Results and Discussion

The benzylation of 6-aminopurine proceeded successfully under all investigated conditions; however, the direction and efficiency of substitution strongly depended on the reaction medium and the base used. In polar aprotic solvents such as DMF and DMSO, the reaction occurred faster and gave higher yields compared with ethanol. This behavior

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is explained by improved stabilization of the purine anion and enhanced nucleophilicity of ring nitrogen atoms in aprotic media. When potassium carbonate was used as a mild base, the reaction predominantly produced the N9-benzylated derivative, whereas stronger bases such as sodium hydride promoted partial formation of the N7-substituted isomer. Thus, regioselectivity of alkylation was controlled mainly by deprotonation strength and solvation effects. Increasing temperature accelerated conversion but did not significantly change the substitution pattern **Table 1**.

Table 1. Effect of reaction conditions on benzylation of 6-aminopurine

№	Solvent	Base	Temperature (°C)	Reaction time (h)	Main product	Yield (%)	N7/N9 ratio
1	Ethanol	K_2CO_3	78	8	N9-benzyl-6-aminopurine	52	10/90
2	DMF	K_2CO_3	75	5	N9-benzyl-6-aminopurine	71	15/85
3	DMSO	K_2CO_3	70	5	N9-benzyl-6-aminopurine	74	20/80
4	DMF	NaH	65	4	Mixture (N7 + N9)	78	45/55
5	DMSO	NaH	60	4	Mixture (N7 + N9)	81	50/50
6	DMF	Et_3N	80	7	N9-benzyl-6-aminopurine	63	18/82

Spectroscopic analysis confirmed formation of benzylated purine derivatives. In the FT-IR spectra disappearance of the N–H stretching band corresponding to the imide nitrogen

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and the appearance of additional aromatic C–H vibrations indicated successful substitution. The UV–Vis spectra showed bathochromic shifts of absorption maxima, reflecting extension of conjugation after introduction of the benzyl fragment. The ^{1}H NMR spectra displayed characteristic signals of benzyl CH_2 protons and aromatic ring protons together with changes in chemical shifts of heterocyclic protons, allowing identification of substitution position. These data demonstrated that alkylation modified the electronic density distribution in the purine system and increased molecular hydrophobicity.

The obtained benzyl derivatives were subsequently immobilized into hydrogel capsules. During swelling of the polymer matrix, the compounds diffused into internal pores and were retained inside the network. Encapsulation efficiency depended on the degree of crosslinking and hydrophilicity of the gel. Moderately cross-linked hydrogels exhibited the highest loading capacity because they possessed sufficient free volume while maintaining structural stability. Highly dense gels limited diffusion, whereas loosely cross-linked gels partially released the compound during washing.

Release experiments revealed a gradual diffusion-controlled behavior. An initial slow stage corresponded to hydration and relaxation of the polymer network, followed by steady release of the active compound into aqueous medium. The absence of a rapid burst effect indicated strong intermolecular interactions between the purine derivative and hydrogel functional groups, mainly hydrogen bonding between amino and carbonyl groups. Benzyl substitution increased retention time compared with non-substituted adenine, confirming that hydrophobic fragments enhance affinity to the polymer matrix.

The combined results demonstrate a clear relationship between molecular structure and delivery behavior. Regioselective benzylation not only modifies spectral and physicochemical properties of 6-aminopurine but also significantly improves its immobilization stability inside hydrogel carriers. Such hybrid systems are capable of providing prolonged and controlled release of biologically active purine derivatives, which is especially important for applications requiring sustained activity in aqueous environments, including agricultural growth regulation and stress-protective treatments.

The results show that mild bases favor N9 substitution, while stronger bases increase formation of the N7 isomer. Polar aprotic solvents improve reaction yield and conversion efficiency.

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Conclusion

This work demonstrated that 6-aminopurine undergoes efficient benzylation under alkaline conditions, and the direction of substitution is governed primarily by the base strength and solvent polarity. Polar aprotic media favored higher conversion, while mild bases led mainly to N9-benzylation and stronger bases promoted formation of both N7 and N9 isomers. Spectral analysis confirmed successful structural modification and indicated redistribution of electron density within the purine ring after introduction of the benzyl group.

The benzylated derivatives were successfully immobilized into hydrogel capsules via swelling–diffusion incorporation. The polymer matrix provided stable retention of the compounds through hydrogen bonding and weak intermolecular interactions, resulting in high loading efficiency. Release experiments showed gradual diffusion-controlled liberation of the active substance without an intensive burst stage.

Thus, benzylation improves compatibility of 6-aminopurine with hydrogel carriers and enables formation of stable hybrid systems capable of prolonged release. The obtained materials may serve as controlled-delivery forms of biologically active purine derivatives suitable for long-term functional applications, particularly where sustained activity in aqueous environments is required.

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