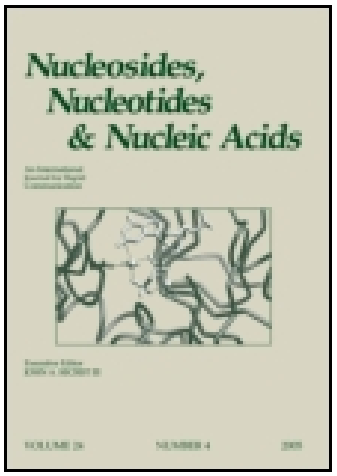

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Poly(ADP-Ribose)—A Unique Natural Polymer Structural Features, Biological Role and Approaches to the Chemical Synthesis

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POLY(ADP-RIBOSE)—A UNIQUE NATURAL POLYMER STRUCTURAL FEATURES, BIOLOGICAL ROLE AND APPROACHES TO THE CHEMICAL SYNTHESIS

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□ *Poly(ADP-ribose) (PAR) is a natural polymer, taking part in numerous important cellular processes. Several enzymes are involved in biosynthesis and degradation of PAR. One of them, poly(ADP-ribose)polymerase-1 (PARP-1) is considered to be a perspective target for the design of new drugs, affecting PAR metabolism. The structure of PAR was established by enzymatic hydrolysis and further analysis of the products, but total chemical synthesis of PAR hasn't been described yet. Several approaches have been developed on the way to chemical synthesis of this unique biopolymer.*

Keywords DNA alkylation; nucleotide excision and repair; bioconjugates; nucleic acid biosynthesis; modified nucleosides; enzyme inhibition

Poly(ADP-ribose) (PAR) participates in numerous cellular processes such as DNA repair and replication, modulation of chromatin structure, transcription, cell differentiation and also in pathogenesis of various diseases such as cancer, diabetes, ischemia and inflammations.[1–3] PAR is the last one among important natural biopolymers, which has not been chemically synthesized. The development of methods for the synthesis of PAR is still a challenge for chemists. A biological function of PAR may be associated with its unique structure, bearing the most negative charge per monomeric unit among all known natural biopolymers. Ionic interactions between negatively charged PAR and positively charged proteins play an important role in the formation of nucleoprotein complexes.[20]

In the previous reviews [1–2, 4–9] biochemical, biological, and some medicinal aspects of PAR functions were described in details. The present review

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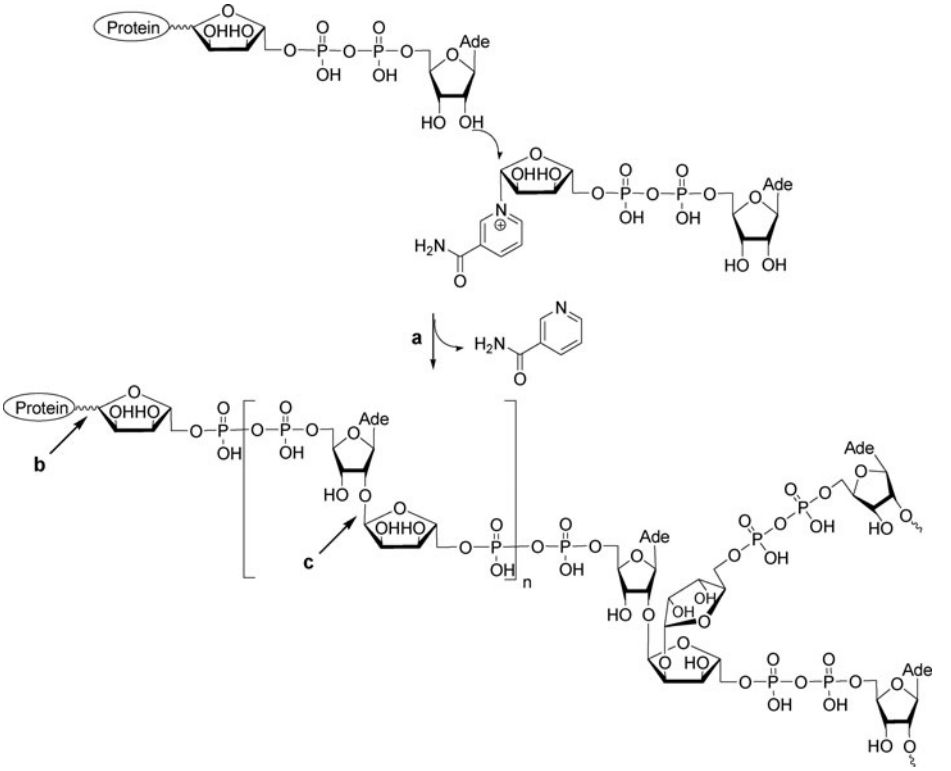
summarizes structural peculiarities, physicochemical properties, and perspectives in chemical synthesis of PAR.

1. DISCOVERY AND STRUCTURE ELUCIDATION OF PAR

Poly(ADP-ribosylation) is a posttranslational modification of proteins in eukaryotic cells, catalyzed by poly(ADP-ribose)polymerases (PARPs). These enzymes promote the conversion of NAD^+ to poly(ADP-ribose) (PAR) with the release of nicotinamide (Scheme 1).[3]



SCHEME 1 Structure and biosynthesis of PAR: a. poly(ADP-ribose) polymerase-1 (PARP). b. (ADP-ribosyl)proteinlyase. c. poly(ADP-ribose) glycohydrolase (PARG).

PAR is a complex branched biopolymer, in which 2′-*O*-α-D-ribofuranosyladenosine moieties are bound by pyrophosphate linkages (Scheme 1). PAR molecules either isolated from natural sources or synthesized in vitro contain more than 200–300 monomeric units.[1, 3] Linear segments of 20–50 units alternate with branched fragments.[1, 3, 10, 13] The chemical structure of PAR was determined by enzymatic hydrolysis of the pyrophosphate bonds by phosphodiesterase with subsequent enzymatic dephosphorylation by alkaline phosphatase and further characterization of the products by NMR

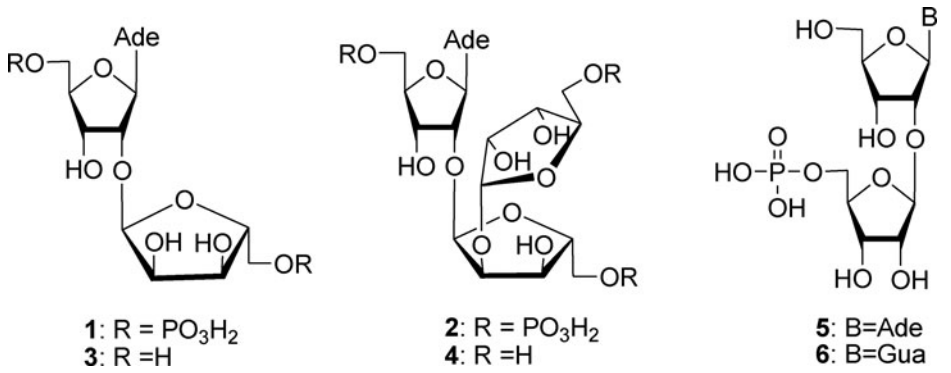


FIGURE 1 Structural fragments of PAR (**1-4**) and 2′-*O*-(5″-*O*-phospho-*β*-D-ribofuranosyl-1-yl)nucleosides (**5-6**)—the closest natural analogues of **1**.

spectroscopy.[3, 10, 13] Enzymatic hydrolysis of PAR by snake venom phosphodiesterase yielded disaccharide nucleoside[11, 10, 13] and trisaccharide nucleoside[10] diphosphates **1** and **2** (Figure 1). Nucleoside diphosphates **1-2** were hydrolyzed by alkaline phosphatase to corresponding nucleosides **3** and **4**.[11]

To date, about a hundred disaccharide nucleoside derivatives have been isolated from various natural sources.[3, 10] 2′-*O*-*β*-D-ribofuranosyladenosine Arp (**5**) and its guanosine derivative—Grp (**6**) were isolated from tRNA.[12-14] The greatest difference in ^{1}H-NMR spectrum of monomeric unit **1** in comparison to 2′-*O*-(5″-*O*-phospho-*β*-D-ribofuranosyl-1-yl)-adenosine **5,** the closest natural analogue of **1**, is observed in coupling constants of the extra carbohydrate moiety due to the difference in configurations of C-1″ atoms. Configuration of *O*-glycosidic bond in isolated nucleosides was unambiguously established by comparative analysis of ^{13}C-NMR spectra of **2** and anomeric methyl-D-ribofuranosides.[10] These compounds contain an extra carbohydrate residue linked to one of the nucleoside hydroxyl groups *via* an *O*-glycosidic bond. The presence of a disaccharide residue and a heterocyclic base makes their properties similar to those of carbohydrates and nucleosides.[3]

The ability of forming helicoidal secondary structure by long-chain PAR has much in common with the structure of DNA and RNA.[1] PAR can form regular helical structures, stabilized by base stacking and hydrogen bonding interactions.[15–16] Although PAR exists in an extended conformation in low salt buffers, it may undergo some structural changes in high salt buffers, showing cooperative helix-coil transitions with hypochromicity up to 40% for long chain molecule.[15] The rise of ionic strength of solution leads to more intensive Cotton effects for long chain PAR polymers. Melting curves monitored by CD-spectra at high salt concentrations have sharp transitions

with T_m values increasing from 20 to 40°C upon the rise of ionic strength from 3 M to 5 M NaCl, respectively.[15]

Recently, an efficient one-pot enzymatic synthesis of ^{13}C, ^{15}N-enriched PAR with 8% overall yield was performed by Schultheisz and co-workers.[16] The isotopically labeled PAR was studied by the NMR methods. According to the NOESY-data adenine base is characterized by anti-conformation. Heterocyclic moieties in PAR are accessible both for inter- and intramolecular interactions, that is in agreement with possibilities of helix-coil transitions, established by CD-spectroscopy.[16] Three-dimensional structural information available by investigations of isotopically labeled PAR provides possibility for modeling and prediction of PAR complexation with proteins.

In cell, PAR is covalently linked to PARPs, histones, and other nuclear proteins.[1] Covalent binding of target proteins with PAR is not specific. Enzymatic modification proceeds either on backbone carboxyl groups of glutamic/aspartic acid residues (formation of ester linkages) [17] or on lysine ε-amino groups.[18–19] The site of poly(ADP-ribosylation) was determined by enzymatic hydrolysis of long PAR-fragments by ADP-ribosylhydrolase (ARH3) and further analysis of ADP-ribosylated peptides by electron transfer dissociation.[18] Modification of proteins by ADP-ribose can also be characterized according to their chemical properties: ADP-ribosylated lysine residues are stable in 1M hydroxylamine at pH 7, while glutamic and aspartic acid residues are easily cleaved under such conditions, releasing ADP-ribose fragments.[20]

2. ENZYMES OF PAR METABOLISM

Several enzymes are involved in biosynthesis and degradation of PAR (Scheme 1). In cellular nuclei PAR is synthesized by PARPs (scheme 1, pathway a) from NAD^+ as a substrate. At present time about 17 enzymes of PARP family are known, among them are PARP-1, PARP-2, PARP-3, tankyrases etc. The properties of the most enzymes from PARP family have not been thoroughly studied yet.

PARP-1 (EC 2.4.2.30) is responsible for the synthesis of 90% of PAR in cell[21] and appears to be the major poly(ADP-ribosyl)ating enzyme in higher eukaryotes after DNA-damage. PARP-1 assists in the repair of single-strand DNA nicks by base excision repair (BER) pathway in mammals[8] and is defined as a protein, catalyzing PAR formation in response to genotoxic stress or mitogenic stimuli.[1, 17] Apart from its role in BER, PARP-1 is involved in modulation of chromatin structure, DNA-replication, gene transcription, repair of DNA double strand breaks (DSB), restarting of collapsed replication forks and genomic maintenance possibly by interaction with p53.[1, 8, 22, 23] PARP-1 consists of three main domain structures: DNA-binding domain (DBD) with molecular mass 46 kDa, automodification domain (AMD) with

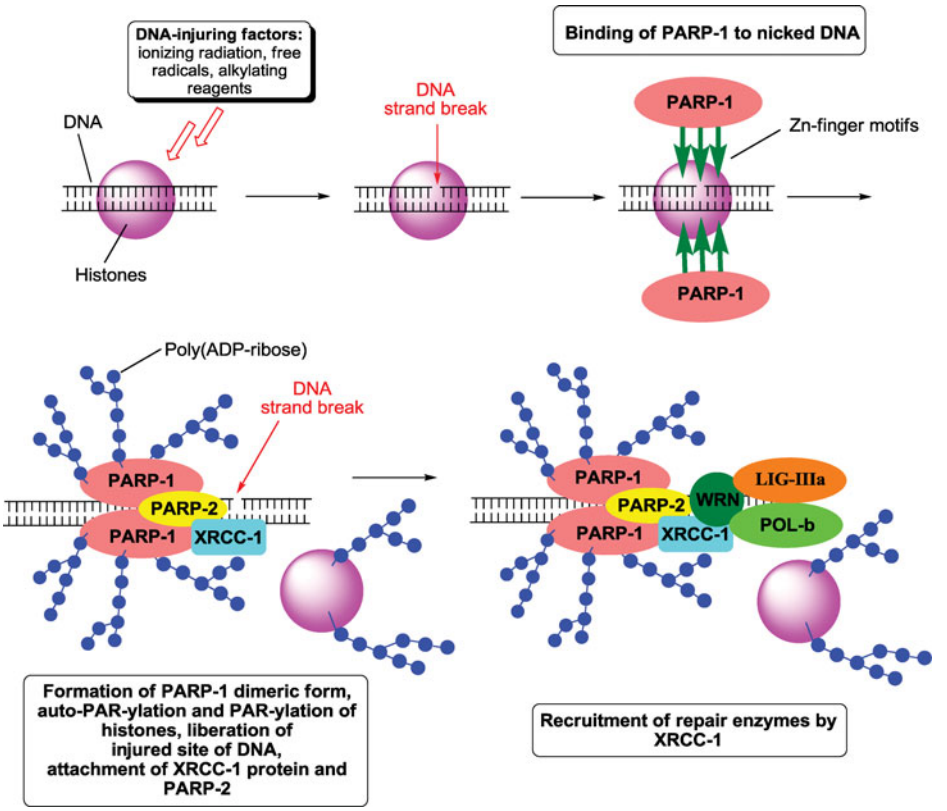
molecular mass 22 kDa and catalytic domain with molecular mass 54 kDa (cat-PARP).[2]

DBD includes nuclear localization signal (NLS)[8] and three zinc finger motifs FI, FII [1, 8–9] and FIII.[8–9] FI and FII recognize damaged DNA structure [1, 6, 9] and are important for the binding to single- and double-strand breaks (SSB and DSB).[1, 6, 8] FIII participates in alterations of PARP-1 catalytic activity in response to the coupling with damaged DNA [8–9], as a result the catalytic activity increases up to 500-fold resulting in PAR-ylation of variety of proteins during 15 to 30 s after damage.[8] AMD, which is necessary for regulation of PARP-1 catalytic activity, is located in the central part of PARP and contains acceptor aminoacids for covalent attachment of PAR.[4] AMD also contains leucine-zipper motif at N-terminal region [1], which is suggested to be involved in the dimerization of PARP and complexation with other nuclear proteins.[1] Recent studies revealed, that enzymatic activity of RARP-1 is regulated only by automodification of acceptor lysine residues (Lys-498, 521, and 524) located in AM-domain.[20]

Cat-PARP is responsible for PAR biosynthesis and implements at least three enzymatic reactions [1]: (a) initiation (the attachment of the first ADP-ribose moiety to an acceptor amino acid); (b) elongation of PAR chain by joining of additional ADP-ribose monomers; (c) generation of branching points.

Active site of catalytic domain consists of two sub-domains: acceptor sub-domain for binding adenosine and donor sub-domain for binding nicotinamide residue of NAD^+ Aminoacid sequence in cat-PARP is conserved and insignificantly differs for different species of higher eykariotes.[2] The mechanism of poly(ADP-ribosylation) has not been thoroughly investigated yet. A multipoint fixation of NAD^+ at nicotinamide, pyrophosphate, and adenosine moieties is supposed to direct 2′-*O*-hydroxyl group for nucleophilic attack on C-1′ carbon atom with the following release of nicotinamide. It has been shown that base modified NAD-analogues: 1,N^6-etheno-NAD^+(ε-NAD^+), nicotiamide guanine dinucleotide (NGD^+), nicotinamide hypoxantine dinucleotide (NHD^+) are rather poor substrates for PARP-1 as compared to NAD^+, leading to short-chain modified molecules of PAR.[24] Nevertheless, modified NAD-analogues open possibilities of fluorescent labeling of substrate proteins of PARPs, for example, by «click chemistry» [25], other NAD-consuming enzymes[26], detection of membrane-associated proteins and even for visualization of organelles, cells, and tissues.[27]

Ionic interactions with target proteins underlie in functional role of PAR.[28] When linked to various target proteins negatively charged PAR significantly changes their properties.[29] PAR-ylation of PARP-1 itself is a general pathway of enzymatic activity regulation.[20] A series of proteins participating in DNA synthesis and repair, transcription, modulation of chromatin structure, cell cycle are recognized by PARP-1 as specific targets.[1, 28] A wide range of proteins are involved in DNA repair, particularly—histones [1, 28],



SCHEME 2 Formation of repair complex on nicked DNA.

X-ray repair cross-complementing proteins (XRCC) [28], DNA-polymerase β [1, 28], catalytic subunit of DNA-polymerase ε, double strand-break repair protein MRE-11, DNA ligases I and III, DNA mismatch repair protein MSH2, DNA-topoisomerases, DNA-dependent protein kinase catalytic subunit etc.[28] Lysine and arginine-containing motifs in these proteins are involved in ionic interactions with PAR.[28] It was shown, that binding of protein depends on molecular weight of PAR. For example p53 manifests ability to form complexes with long PAR chains (>40 units), while protein XPA can bind to short (20–40 units) and long (>40 units) PAR chains.[22] In solution one protein can form several complexes with PAR of different molecular weights.[22]

Scheme 2 summarizes literature data on interactions of different proteins, participating in DNA repair.

Ionizing radiation, free radicals, and alkylating agents cause modification of heterocyclic bases in DNA. The excision of modified base by DNA-glycosylases and further hydrolysis of injured DNA site by AP-endonucleases lead to the nick in the DNA-strand, named single-strand break

TABLE 1 Composition of repair complexes under two different BER-mechanisms

Type of repair	Participating proteins
Short patch repair (single strand break)	PARPs-1/2, XRCC-1, DNA-polymerase-β (POL-β), Ligase-IIIα (LIG-IIIα), WRN (helicase)
Long patch repair (multiple single strand breaks)	PARPs-1/2, XRCC-1, DNA-polymerases-δ/ε (POL-δ/ε), Ligase-I (LIG-I), WRN, Flap-endonuclease-1 (FEN-1), Proliferating cell nuclear antigen (PCNA)

(SSB). After activation by SSB PARP-1 homodimer poly(ADP-ribosyl)ates histones, facilitating uncoiling of nicked DNA from modified histones by electrostatic repulsion.[29–31] X-ray repair cross-complementing I protein (XRCC-1) has high affinity to PAR and serves as a scaffold for binding with the repair enzymes and construction of the repair complex[28, 31] (Table 1).

Protein repair complexes under short patch repair and long patch repair are quite different.[31] PARPs, XRCC-1, DNA-polymerase-β (POL-β), and ligase-IIIα participate in short patch repair. Long-patch repair complex contains DNA-polymerases-δ/ε instead of DNA-polymerase-β, ligase-I instead of ligase-IIIα and also contains flap-endonuclease-1 and protein PCNA that acts as a processivity factor for DNA-polymerase-ε. It has been shown that enzyme PARP-2, attached to PARP-1, accelerates DNA repair. PARP-1 in complex with PARP-2 works more effectively than single PARP-1.[31] In fact the scheme, described here, is simplified, because PARP-1 is involved in many cellular processes and can recruit hundreds of proteins bearing specific sites for binding with PAR.[8]

As PARP-1 is involved in DNA repair mechanism, it is considered as the perspective enzymatic target for design of new drugs for the treatment of cancer, ischemia and some other diseases.[2, 32–34] It has been shown that breast cancer-associated genes *BRCA1* and *BRCA2* play an important role in the repair of double strand breaks (DSB) through homologous recombination, suppressing tumor growth.[8, 33] The loss of one gene function (PARP-1 or BRCA1/2) is not dramatic event for the cell, but the loss of both genes (PARP-1 and BRCA1/2) is lethal. In BRCA1/2 deficient cells PARP-1 is upregulated. In this case, PARP-1 inhibitors can be used as single agents for the treatment of BRCA-deficient tumors, such as breast and ovarian cancer.[8, 33] PARP-1 inhibition is a reliable diagnostic test for identification of BRCA-deficient forms of cancer and for effectivity of PARP inhibition therapy.[33] The use of alkylating agents in chemotherapy of cancer leads to DNA damage both in healthy and cancer cells. Therefore the system of DNA-repair is being activated in both cases. Inclusion of PARP-1 inhibitors into the complex chemotherapy is expected to decrease concentration of

TABLE 2 Current clinical trials of PARP-1 inhibitors*

Compound	Company	Single agent/ combination	Therapeutic indication	Phase (2014)
Rucaparib	Pfizer	Single agent	Solid tumors, pancreatic cancer	2
		Single agent	Ovarian cancer	3
BMN-673	BioMartin	Single agent	Hematological tumors	1 (terminated)
		Combination with temozolomide (TMZ)	Solid tumors	2
GPI21016/E7016	MGI-Pharma/Eisai	Combination with TMZ	Solid tumors	1 (completed)
		Combination with TMZ	Melanoma	2
Veliparib	Abbott	Combination with hemcytabine hydrochloride and cisplatin	Adenocarcinoma and pancreatic cancer	2
		Single agent	Solid tumors	1 (completed)
Olaparib	AstraZeneca	Single agent	Solid tumors	1
INO-1001	Inotek/Genetech	Combination with TMZ	Melanoma	1 (terminated)
Niraparib	Merck	Combination with TMZ	Solid tumors	1
CEP-8983	Cephalon	Single agent	Solid tumors	1-2 (terminated)

*http://www.clinical trials.gov (a service of the U.S. National Institutes of Health)

alkylating agents and, hence, to decrease the toxicity of the therapy. PARP-1 inhibitors can be used for the treatment of different forms of cancer in combination with chemotherapeutic agents and also in monotherapy (Table 2).

A wide range of natural compounds are known to inhibit PARP-1: nucleosides, purines and pyrimidines, vitamins (A, K, F), endocrine substances, taurine, antibiotics, metal ions (Zn^{2+}, Cd^{2+}, Hg^{2+}, Cu^{2+}, Cu^{+}, Co^{2+}, Ni^{2+}).[35] Substituted benzamides were the first known synthetic analogues of nicotinamide and first inhibitors of PARP-1, competing with NAD^{+} for binding with PARP-1.[34] Further experiments have led to the discovery of new classes of inhibitors based on analogues of 3-aminobenzamide with nanomolar inhibitory constants [33–34, 36] (Figure 2).

The most important structural motifs in PARP-1 inhibitors are carboxamide fragment and aromatic moiety.[33, 36] To improve some characteristics of PARP-1 inhibitors auxiliary pharmacophores, ionogenic groups or hydrophobic moieties are attached to the molecule of inhibitor *via* linker of variable length. Attachment of such functionalized substituents may increase solubility in water or selectivity of accumulation in cells or ability to form stable complexes with target enzyme PARP-1.[33]

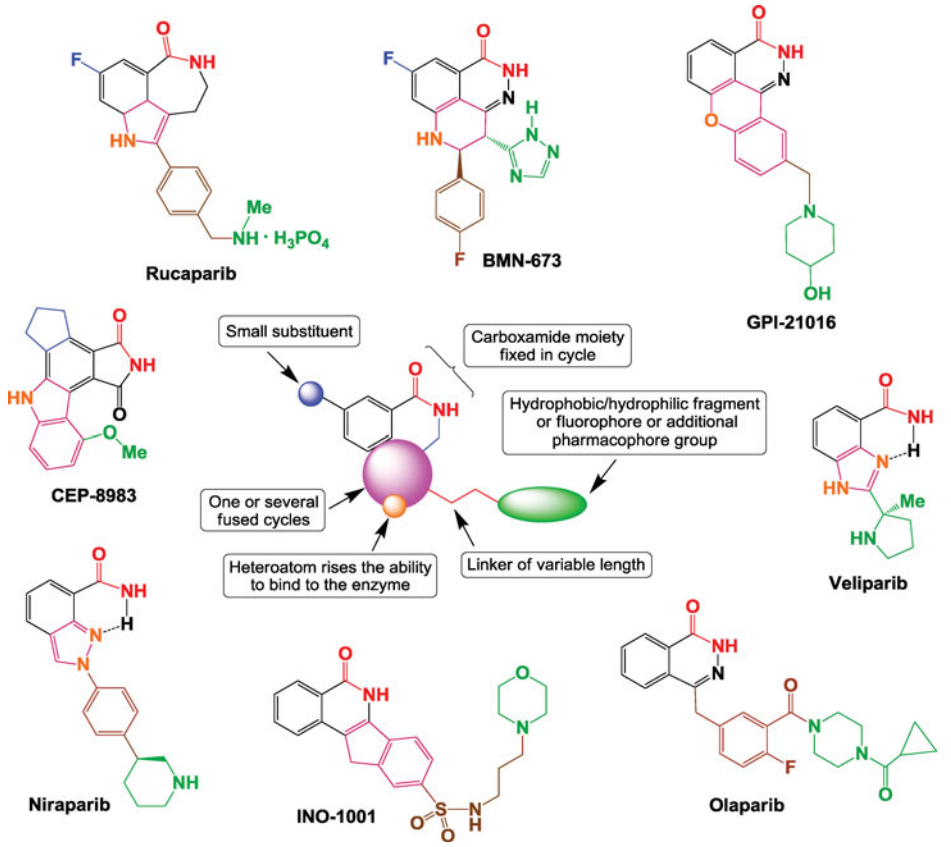


FIGURE 2 Structural motifs in lead PARP-1 inhibitors.[33–34]

Natural nucleosides and nucleotides are less active toward PARP-1 than polycyclic analogues of 3-aminobenzamide ($IC_{50} \sim 200\ \mu M$).[35] Their properties can be improved by chemical modifications. Disaccharide nucleosides seem to be advantageous class of PARP-1 inhibitors due to their cell penetrability and low cytotoxicity.[37]

It has been shown, that many of PARP-1 inhibitors, even the drugs for clinical trials, possess cross-binding affinity to more than one member of PARP family, thus demonstrating polypharmacology in PARP-inhibition.[29] To better understand the results of biochemical and clinical studies and also to avoid the risks, associated with broad-range PARP-inhibition, novel selective PARP inhibitors are required. Biological activity of the most known PARP-1 inhibitors is based on interaction with the target in the nicotinamide pocket of the enzymatic active site.

Nowadays, several routes to new compounds are proposed: (a) targeting the putative adenine binding site and (b) targeting the D-loop of catalytic subunit, which is variable in structure and in sequence.[29]

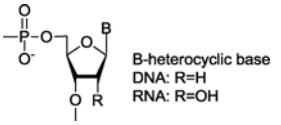
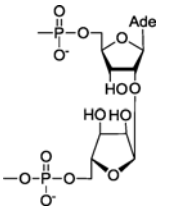
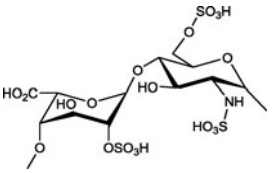
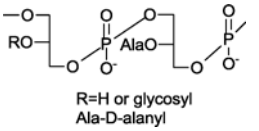
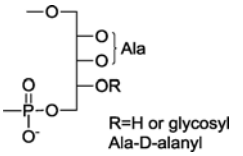
Biochemical degradation of PAR is mediated by two enzymes, *viz.*, ADP-ribosyl protein lyase (scheme 1, pathway b) and poly(ADPribose) glycohydrolase (PARG) (scheme 1, route c)[17], playing the major role in stopping of DNA repair system. *O*-Glycoside bond between adenosine and ribofuranose moieties in PAR-polymer is split by PARG. The structure of PARG and several functions, carried out by PARG in cell, are still not well-studied because of increased sensitivity of this enzyme to proteolysis.[17, 38] PARG exists in two isoforms[38]: full-sized enzymatic form (110 kDa) is accumulated in nucleus, while short form of enzyme (85 kDa) prevails (85% from total quantity of PARG) and is localized in cytoplasm, Golgii apparatus and endoplasmatic reticulum. *N*-terminal part of enzyme is supposed to regulate PARG localization in cell compartments.[38] Preferable localization of PARG in cytoplasm is likely necessary for cell protection, because free PAR is shown to be toxic and can cause cell death, penetrating from nucleus to cytoplasm.[39] Besides DNA repair and regulation PARP-1 activity in cell nucleus PARG also participates in DNA replication, carrying out the recovery of initial structure (chromatin recondensation).[17] It was also hypothesized that PARG participates in apoptosis.[38]

ADP-ribosyl protein lyase catalyzes the removal of proximal adenine-diphosphoribosyl (ADPR) residue from proteins but does not cleave poly(ADP-ribosyl)ated proteins.[17, 40] ADPR-hydrolases (ARH), enzymes, splitting single ADPR-moieties and short PAR fragments are also known.[41] Earlier studies indicated that PARG can also function as lyase and catalyze cleavage of ADPR residues linked to carboxylate groups of histone H1.[17]

3. NEGATIVELY CHARGED NATURAL BIOPOLYMERS AND PAR

In PAR polymer disaccharide units are joined by pyrophosphate linkage, that makes PAR the most electronegative biopolymer (in pyrophosphate $pK_1 < 2.0$, $pK_2 = 2.64$ [42]). Other biopolymers, such as DNA and RNA, contain only one phosphate group per monomeric unit and have only one negative charge at neutral pH. Heparan sulfate and heparin belong to the glycosaminoglycan polymers built from repeating disaccharide units. Their main disaccharide unit is shown in Table 3. The presence of sulfate and carboxyl groups defines the substantial negative charge of these biopolymers under neutral conditions. In heparin, such groups reach an average of 2.7 on the disaccharide unit. The main difference between heparin and heparan sulfate is the lower degree of sulfonation of the later.[43] Teihoic acids isolated from cell wall of gram-positive bacteria consist of repeating polyol or glycosylpolyol residues joined together with phosphodiester linkages.[44]

TABLE 3 Some negatively charged biopolymers

Polymer	Structure of monomeric unit	Ionogenic group	Charge per monomeric unit at neutral pH
DNA or RNA		Phosphate	1
PAR		Pyrophosphate	2
Heparin		Sulfate, carboxyl*	1.35*
Glycerol teihoic acid		Phosphate	1**
Ribitol teihoic acid		Phosphate	1

*2.7 on disaccharide unit; **2 on diglycerol unit.

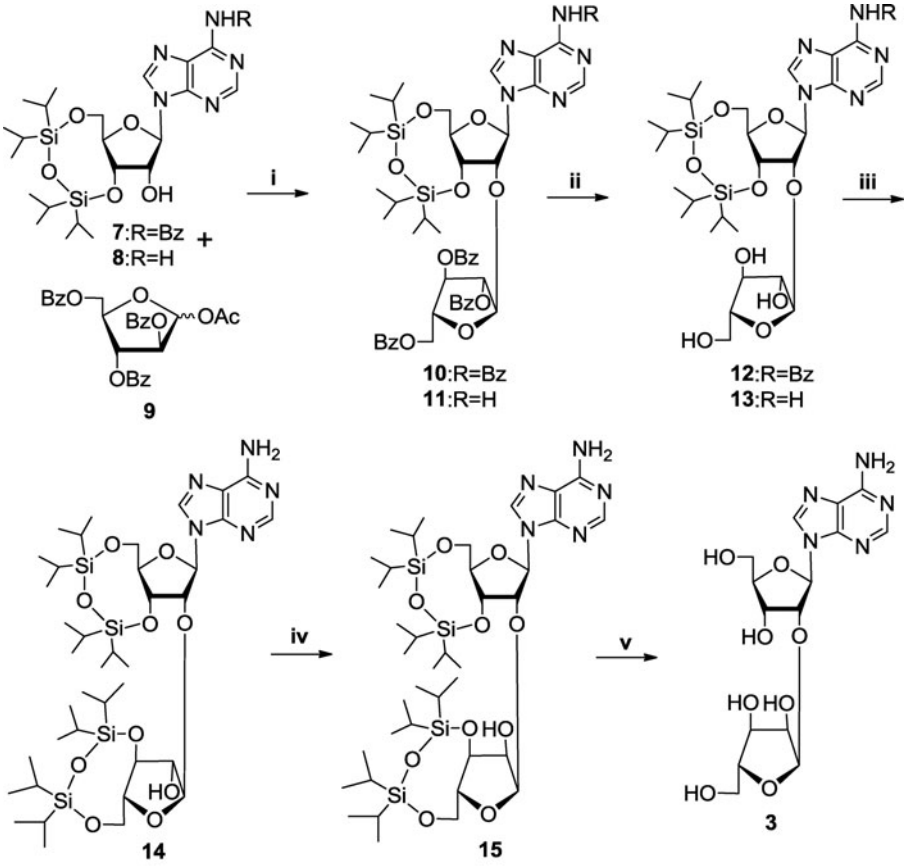
4. APPROACHES TO THE CHEMICAL SYNTHESIS OF PAR

The development of methods for the synthesis of PAR is still a challenging problem for chemists and the first obvious step on this way is the synthesis of disaccharide monomeric unit of PAR. To Oligonucleotides containing disaccharide nucleosides have been obtained so far.[3] However, the formation of pyrophosphate internucleoside linkage remains the major problem. Trisaccharide nucleoside, located in the branching points of PAR, also hasn't been obtained by chemical approaches.

4.1. Synthesis of Disaccharide Nucleosides

Disaccharide nucleosides can be synthesized by one of two routes. These consist of coupling of a protected disaccharide with a heterocyclic base derivative or the formation of an *O*-glycosidic bond between a nucleoside carrying one free hydroxy group and an activated monosaccharide. Obviously, the use of available natural carbohydrates and nucleosides can significantly shorten the synthetic procedure.[3]

Recently 2′-*O*-α-D-ribofuranosyladenosine, a monomeric unit of PAR, was obtained for the first time.[45] The strategy, based on the inversion



Reagents and conditions: **i.** $SnCl_4$/DCE, 0°C, 24 h, **7**→**10**: 62%, **8**→**11**: 64%; **ii.** 0.1 M MeONa/MeOH, 10°C, 40 min, **10**→**12:** 47% or 8 M $MeNH_2$/EtOH, 20°C, 24 h, **10**→**13**: 77%, **11**→**13**: 87%; **iii.** $TiPDSCl_2$ /pyridine, 24 h, 20°C, **13**→**14:** 80%; **iv.** DMSO/Ac_2O, 65°C, 4 h, then—$NaBH_4$/EtOH, 0°C, 1h, 62%; **v** $Bu_4NF{\cdot}3H_2O$/THF, 20°C, 1 h, 69% or $Et_3N{\cdot}HF$/THF, 20°C, 24 h, 78%.

SCHEME 3 Synthesis of 2′-*O*-α-D-ribofuranosyladenosine.

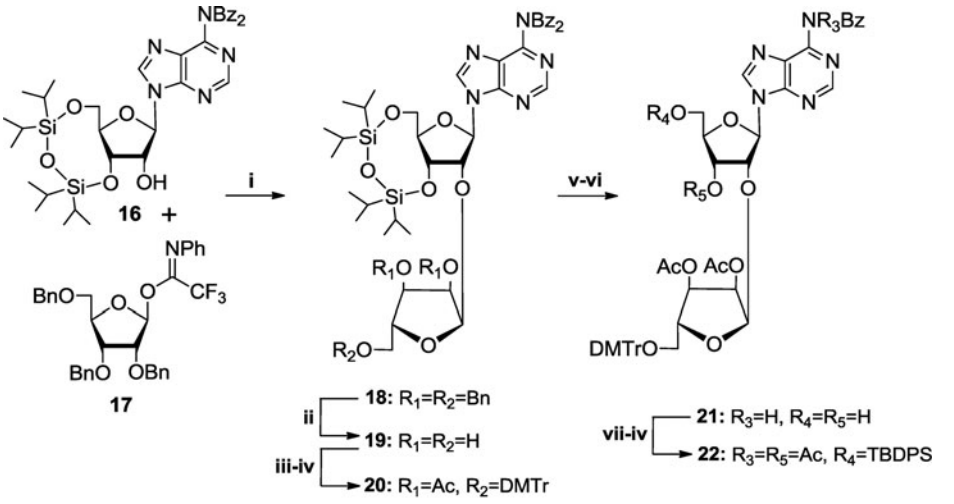
of configuration at C-2 of additional monosaccharide residue in 2′-*O*-α-D-arabinofuranosyladenosine **14,** was chosen (Scheme 3). The initial step was the condensation of TIPDS-protected adenosine **7**, carrying one free hydroxyl group, with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-α/β-D-arabinofuranose **9** (Scheme 3, conversion i).

The use of sodium methoxide afforded *O*-debenzoylation (Scheme 3, conversion ii) but N^6-benzoyl derivative **12** was isolated with only 47% yield due to the partial desilylation. The use of alkylamines in ethanol for deacylation of 3′,5′-*O*-TIPDS-protected nucleosides provided more mild conditions as compared with sodium methoxide, and helped to avoid partial desilylation of TIPDS-protecting group, essentially increasing the yield of deprotection stage.[46]

Inversion of configuration at C-2 of the extra monosaccharide residue in **14** was conducted in two steps—oxidation of the 2″-hydroxyl group to a ketone (not shown) and its reduction with sodium borohydride (Scheme 3, conversion iv).

The use of partially protected nucleoside **8** (R = H) and methylamine in methanol allowed the increase of the overall yield of the resulting product **3** from 13 to 21%.[47]

Recently, an approach for the preparation of divergently protected 2′-*O*-α-D-ribofuranosyladenosine for the synthesis of various ADP-ribosylated peptides and PAR oligomers was developed (Scheme 4).

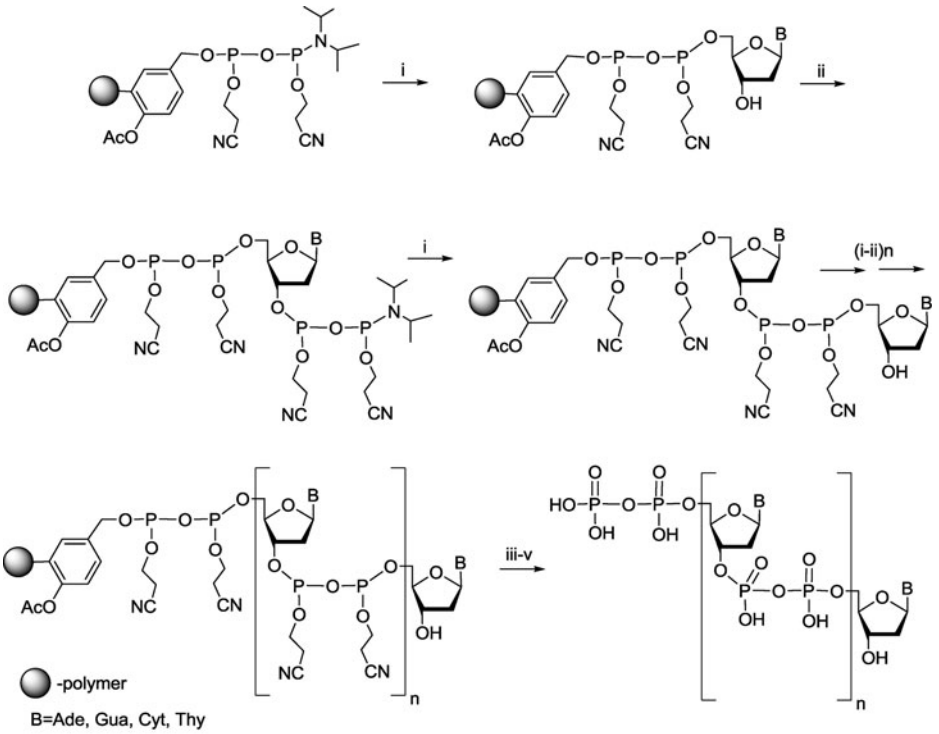


Reagents and conditions: **i.** TMSOTf, 60%; **ii.** BCl_3, −78°C, 45%; **iii.** DMTrCl/Py; **iv.** Ac_2O/Py, 55% (**iii**+**iv**); **v.** $MeNH_2$, 0°C; **vi.** Bu_4NF/THF; **vii.** TBDPSCl/Py, 49% (**v**+**vi**+**vii**+**iv**).

SCHEME 4 Synthesis of protected 2′-*O*-α-D-ribofuranosyladenosine.

Condensation of adenosine derivative **16** with 1-(*N*-phenyl)-2,2,2-trifluoroacetamido-2,3,5-tri-*O*-benzyl-β-D-ribofuranose (**17**) in the presence of TMSOTf (scheme 4, conversion i) proceeded with preferable formation of α-anomer.[48] Benzyl protecting groups (Bn) were removed by BCl_3 at $-78°C$ (Scheme 4, conversion ii). Further manipulations with protecting groups led to disaccharide nucleoside **22**. However, the yield of 2′-*O*-α-D-ribofuranosyladenosine (**3**), obtained by this method (10%), was lower than in previously described scheme 3.

Recently, the new method for the preparation of 2′-*O*-α-D-ribofuranosylnucleosides have been reported.[49] The described glycosylation of 3′,5′-*O*-TIPDS-protected uridine with 1-thiotolyl-2,3,5-tri-*O*-benzyl-D-ribofuranoside under AgOTf/*N*-iodosuccinimide catalysis proceeded with high yield (82%) of blocked 2′-*O*-α-D-ribofuranosyluridine.

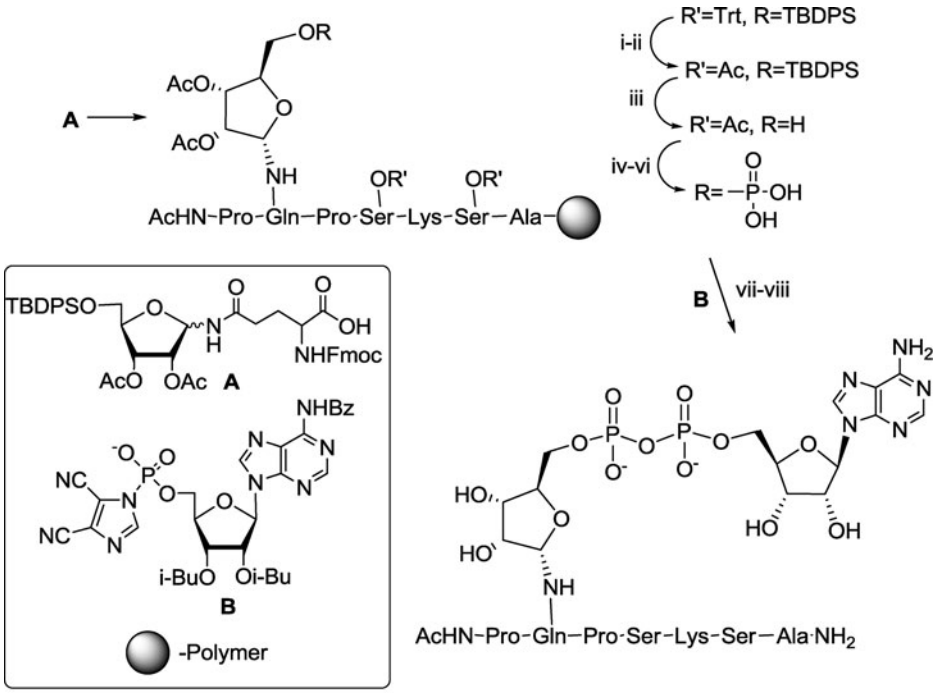


Reagents and conditions: **i.** deoxynucleoside ROH, 1*H*-tetrazole/THF/DMSO; **ii.** [*i*-$Pr_2NPOCH_2CH_2CN]_2O$, THF or THF/DMSO, 1H-tetrazole; **iii.** *t*-BuOOH/THF; **iv.** DBU/THF; **v.** TFA/CH_2Cl_2/H_2O/1,2-ethaneditiol.

SCHEME 5 Pyrophosphate modification of oligodeoxynucleotides.

4.2. Pyrophosphorylation

To date, different methods for the preparation of oligonucleotides, containing disaccharide nucleosides, joined by phosphodiester linkages, have been elaborated.[3, 50–53] Introduction of labile pyrophosphate bond requires more mild conditions. Formation of pyrophosphate linkages by various chemical methods is widely-known and well-described in the literature.[26, 54–57] The synthesis of oligomers, consisting of nucleosides joined by pyrophosphate linkages, was done quiet recently.[58] Amidophosphite method was used for the preparation of such modified DNA-oligomers (Scheme 5).



Reagents and conditions: **i.** 5% CF_3COOH, CH_2Cl_2 **ii.** Ac_2O/DMAP; **iii**—desilylation with HF/pyridine; **iv.** Di(4-methoxybenzyl)-*N*,*N*-diisopropylphosphoramidite/DCI; **v.** *t*-BuOOH; **vi.** 3% aq. CF_3COOH; **vii**—condensation with **B**; **viii.** NH_3/MeOH (deblocking and liberation from polymeric carrier).

SCHEME 6 Solid-phase modification of heptapeptide by ADPR.

Synthesis of modified DNA-oligomers on polymeric carrier didn't require protection of functional groups in nucleosides: sterically hindered diphosphytylating reagent, attached to the polymer, facilitated selectivity of

the reaction at 5′-OH group. After the standard synthetic procedures, including oxidation of pyrophosphites to pyrophosphates, modified oligodeoxynucleotides were released from the polymeric carrier by acidic hydrolysis.

Oligodeoxynucleotides, containing 3′-5′-pyrophosphate internucleotide linkages, formed stable duplexes with complementary DNA strands and haven't been degraded under enzymatic hydrolysis.[58] Expanding the described protocol on PAR oligomers requires divergently protected disaccharide nucleosidic «building unit».

4.3. ADP-ribosylation of Proteins

Peptides containing ADP-ribosyl moiety can be considered as substrates for elongation by PARP-1.[59] Modifications of histones by ADPR may be valuable instrument for investigation of protein interactions with PAR and mechanisms of PARP, PARG, and ADP-ribosylproteinlyase action in vitro. Methods of peptide chemistry can be recruited for such modifications. As an example, ADP-ribosylated isosterical fragment of histone H2B was obtained by this way (Scheme 6).[59]

This fragment is located at the *N*-terminal part of histone and undergoes PAR-ylation during DNA replication or repair.[59] Glutamine, containing ribose moiety (compound **A**), was included into heptapeptide by peptide chemistry. After selective removal of *tert*-butyldiphenylsilyl (TBDPS) protecting group, followed by phosphorylation, the resulting peptide reacted with activated nucleotide **B**. Deprotection and cleavage from the polymeric carrier was carried out in NH_3/MeOH. Peptides, functionalized by aminooxy groups, can be also site-specifically conjugated to ADP-ribose.[60]

CONCLUSION

In a summary, poly(ADP-ribose) (PAR) is a unique biopolymer, participating in numerous key processes in a cell. Its chemical synthesis remains a challenging problem, which hasn't been solved yet. PAR is the most electronegative biopolymer among all known in nature. This property underlies its binding affinity to a wide range of proteins, including PARP-1, which is the major enzyme, catalyzing the synthesis of PAR chains with variable length to regulate the functions of specific proteins and recruit them depending on the cellular event. Understanding of PAR functions in cell requires chemical synthesis of regular PAR oligomers. Some natural sulfated and phosphorylated biopolymers may be considered as PAR mimics.

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