Application Details

Research and Development Minigrants for 2017-2018: Application Review

Application Title: Synthesis of Sulfonamide-Ribonucleoside conjugates as potent antimicrobial, antitumor, or antiviral agents

Application ID: #000060

Review Deadline: Jan 27, 2017 11:59:00 PM

Primary Appointment Title: Assistant Professor of Chemistry

Proposal Summary:

Sulfonamides are an important class of compounds that have been reported as antibacterial, anticancer, and antiviral drugs. At least two clinically used HIV protease inhibitors possess sulfonamide moieties in their molecules, whereas a very large number of other sulfonamide derivatives are constantly being synthesized and evaluated in order to obtain compounds with less toxicity or activity against drug-resistant microbes and viruses. Nucleoside analogs have shown promising results not only as therapeutic agents, but also as potent antimicrobial agents. With deliberate chemical modifications, nucleoside analogs elicit therapeutic effects by inhibiting cancer cell growth and disrupting viral replication. Nucleoside antibiotic, e.g. A201A, with potent antibacterial activities has also been reported. A problem of growing concern is resistance to antibiotics by bacterial pathogens, which increases the demand for newly synthesized antimicrobial agents. In this proposed project, effective methods toward the synthesize of sulfonamide-ribonucleoside conjugated molecules will be investigated. The synthesized molecules will be evaluated concurrently or in a future work for their antimicrobial and anti cancer activities, and their effect will be compared to these of the individual molecules. This proposal will lay groundwork for the pursuit of external funding to develop several nucleoside HIV reverse transcriptase or HIV integrase inhibitors containing sulfonamido groups and to develop sulfonamide-nucleoside analogs as effective therapeutics for treatment of cancer.

Comments to the Administrator(s):

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Ahmed Awad

2010 Via Arandana, Camarillo, CA 93012

Current position: ***Assistant Professor of Chemistry***
California State University Channel Islands
One University Drive, Camarillo, CA 93012

Starting date August 2014

Telephone W: 805-437-2794 / H: 805-482-0240

E-Mail ahmed.awad@csuci.edu

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Biological Chemistry Lectures, Biochemistry Laboratories I and II
SPIRaL UNIV398, Advanced Research Investigations, Therapeutic Nucleic Acids
Bioorganic Chemistry, special topics in chemistry lectures and labs
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References:

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   Address Department of Chemistry
   California State University Channel Islands
   Camarillo, CA 93012
   Phone: 805-437-8999, Email: simone.aloisio@csuci.edu

2. Name: Blake Gillespie
   Address: Professor of Chemistry
   California State University Channel Islands
   Camarillo, CA 93012
   Phone: (805) 437-2796, Email: blake.gillespie@csuci.edu

3. Name: Philip D. Hampton
   Address: Professor of Chemistry
   California State University Channel Islands
   Camarillo, CA 93012
   Phone: (805) 437-8869, Email: philip.hampton@csuci.edu

4. Name: Thomas C. Bruice, Professor
   Address: Department of Chemistry and Biochemistry
   University of California Santa Barbara
   Santa Barbara, CA 93106
   Phone: 805-893-2044 (W); 805-964-2048 (H), FAX: 805-893-2229
   Email: tcbruice@chem.ucsb.edu

5. Name: Marit Nilsen-Hamilton, Professor
   Address: Department of Biochemistry, Biophysics and Molecular Biology
   3206 Molecular Biology Building
   Iowa State University, Ames, IA 50011
   Phone: 515-294-9996, FAX: 515-294-0453, Email: marit@iastate.edu

6. Name: Hartmut Seliger, Professor
Address: Sektion Chemische Funktionen in Biosystemen, Universität Ulm,
Albert-Einstein-Allee-11, 89069 Ulm, Germany.
E-mail: hartmut.seliger@uni-ulm.de
Phone: +49-(0)731-5015180

Research Summary

My research area is in the interface between chemistry and biology. I am interested in investigation of novel drugs for the treatment of cancer. This includes organic synthesis of modified nucleosides, nucleotides and nucleic acids (called therapeutic nucleic acids). The applications of these synthetic molecules in biological systems, as anticancer agents, constitute the major part of my research. I am interested in Pancreatic and Prostate cancer.

In my Ph.D. work, new class of minimally modified oligonucleotides were synthesized, tested towards exo- and endonuclease enzymatic degradation (in model experiments with respective enzymes as well as in human blood serum), and examined with respect to the binding affinity to their complementary DNA and RNA strands. Their use as antisense agents for down-regulation of a specific gene expression was also investigated.

Minimally phosphoramidate backbone modified oligonucleotides were synthesized by a fully automatic solid-phase DNA synthesis, using a combination of phosphoramidite and H-phosphonate methods. 2-(2-Aminoethoxy)ethanol or α,ω-diamino-triethylenglycol was used to introduce a chemically stable, non-charged, long chain, hydrophilic and non-toxic phosphoramidate backbone branches. To still retaining the principle of minimal structure modification, these phosphoramidate modifications were applied only to one, two or three internucleotide linkages of 15mer oligonucleotides. The phosphoramidate moieties were introduced by oxidation of pre-formed H-phosphonate internucleoside bonds with a freshly prepared solution of 0.1 M I_2 and 1.0 M of either 2-(2-aminoethoxy)ethanol or α,ω-diamino-triethylenglycol. The subsequent capping routine introduces a terminal N-acetyl group at the branches in the case of α,ω-diamino-triethylenglycol derivatives. Characterization by MALDI-TOF mass spectrometry confirms the introduction of the phosphoramidate internucleotide linkages.

In the hybridization studies, T_m values of the minimally phosphoramidate backbone modified oligonucleotides with their complementary DNA and RNA sequences were determined under physiological conditions and compared to the corresponding T_m of the native DNA-DNA and DNA-RNA duplexes. A slight depression of the melting point (<1°C per modified nucleotide) was observed, indicating that there is nearly no influence of the phosphoramidate internucleotide linkages derived from 2-(2-aminoethoxy)ethanol or α,ω-diamino-triethylenglycol, when applied only to one, two or three internucleotide linkages, on the binding affinity of their oligonucleotide derivatives to complementary targets DNA and RNA.

In enzymatic studies, the modified oligonucleotides were examined, *in vitro*, with respect to stability towards snake venom phosphodiesterase (as an example for 3`-exonuclease enzymes) and S1 nuclease (as an example for endonuclease enzymes) as well as their degradation in human blood serum (as an example for physiological conditions). These modifications were found to protect oligonucleotides against exo- and endonucleolytic attack. The introduction of only three branches within 15mer oligonucleotide significantly retards the degradation by S1 nuclease. Also, the introduction of such a branches protect oligonucleotides from serum exonucleolyticaly degradation.

The properties of these compounds in gene inhibition were described. 17mer antisense oligonucleotides were designed, targeting K-ras point mutation on the growth of cultured human pancreatic cancer cells (PANC-1). These oligonucleotides were protected by 3`-terminal inversion (INV-oligos) against degradation by 3`-exonucleases, and one to three phosphoramidate internucleotide linkages substituted with α,ω-diamino-triethylenglycol against degradation by endonucleases. Fluorescein group was introduced at the 5`-end to visualize the cellular uptake. The oligonucleotides were synthesized, purified, and transfected into the cells by the liposome-mediated method. Cell-growth activities were estimated by MTT assay, and K-ras p21 protein synthesis was

evaluated with Western blotting. These antisense oligonucleotides were found to inhibit the expression of the mutated K-ras p21 protein in PANC-1 cells. The down-regulation increased with increasing number of branches, with a nearly complete inhibition of the p21 protein biosynthesis with an oligonucleotide containing three phosphoramidate branches.

In my first Postdoctoral position at Iowa State University, I did some research using the antisense technology to identify the regions within a mRNA that are accessible for hybridization. After that I turned to work with aptamers.

Aptamers are nucleic acids that specifically and selectively bind their target molecules. They offer an alternative to monoclonal antibodies for targeting specific proteins. These small RNA or DNA molecules can be selected by *in vitro* procedure called SELEX (Systematic Evolution of Ligands by Exponential Enrichment). We were using aptamers to design a probe to image gene expression *in vivo*. This nucleic acid probe is consisting of three regions. Aptamer, antisense and attenuator regions. In the absence of target mRNA, this probe is closed in a stem loop and the aptamer is inactive. When the mRNA hybridizes with the recognition module, the aptamer is released from the stem and binds the imaging module.

Another *long range goal* of our research is to develop effective reagents for targeted cancer therapy. The proposal focuses on the development of an aptamer-based reagent for prostate cancer. The reagent called "Drugcart" (Drug carrying aptamers for receptor targeting).

In my second Postdoctoral position at University of California, I was working on the multi-step synthesis of modified nucleosides and nucleotides. Then, I used these modified monomers to build the corresponding nucleic acid (oligonucleotide). Testing of these novel anticancer agents in biological systems (as antisense or siRNA) was also considered.

In my current position, in addition to my teaching schedule, I have around seven to nine students every semester enjoying their undergraduate research in my laboratory. We are working on developing methods for preparation of nucleic acid therapeutics called RNG. Replacement of the negatively charged phosphodiester linkages of RNA with positively charged guanidinum linkages provides the polycationic ribonucleic guanidine (RNG). The resulting compounds are designed to be incorporated into standard DNA/RNA synthesis technology. A multi-step synthetic procedure is essential for the preparation of the building blocks, including the isolation of new chemical compounds. Purification and analysis of each product is necessary to determine the chemical structure and to proceed with the next step in the synthetic procedure.

My undergraduate students and myself focus on having a research quality for publications in international journals, in addition to interest in presenting our work at several scientific meetings and conferences.

Ahmed Awad

2010 Via Arandana, Camarillo, CA 93012

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California State University Channel Islands
One University Drive, Camarillo, CA 93012

Starting date August 2014

Telephone W: 805-437-2794 / H: 805-482-0240

E-Mail ahmed.awad@csuci.edu

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Minimally phosphoramidate backbone modified oligonucleotides were synthesized by a fully automatic solid-phase DNA synthesis, using a combination of phosphoramidite and H-phosphonate methods. 2-(2-Aminoethoxy)ethanol or α,ω-diamino-triethylenglycol was used to introduce a chemically stable, non-charged, long chain, hydrophilic and non-toxic phosphoramidate backbone branches. To still retaining the principle of minimal structure modification, these phosphoramidate modifications were applied only to one, two or three internucleotide linkages of 15mer oligonucleotides. The phosphoramidate moieties were introduced by oxidation of pre-formed H-phosphonate internucleoside bonds with a freshly prepared solution of 0.1 M I_2 and 1.0 M of either 2-(2-aminoethoxy)ethanol or α,ω-diamino-triethylenglycol. The subsequent capping routine introduces a terminal N-acetyl group at the branches in the case of α,ω-diamino-triethylenglycol derivatives. Characterization by MALDI-TOF mass spectrometry confirms the introduction of the phosphoramidate internucleotide linkages.

In the hybridization studies, T_m values of the minimally phosphoramidate backbone modified oligonucleotides with their complementary DNA and RNA sequences were determined under physiological conditions and compared to the corresponding T_m of the native DNA-DNA and DNA-RNA duplexes. A slight depression of the melting point (<1°C per modified nucleotide) was observed, indicating that there is nearly no influence of the phosphoramidate internucleotide linkages derived from 2-(2-aminoethoxy)ethanol or α,ω-diamino-triethylenglycol, when applied only to one, two or three internucleotide linkages, on the binding affinity of their oligonucleotide derivatives to complementary targets DNA and RNA.

In enzymatic studies, the modified oligonucleotides were examined, *in vitro*, with respect to stability towards snake venom phosphodiesterase (as an example for 3`-exonuclease enzymes) and S1 nuclease (as an example for endonuclease enzymes) as well as their degradation in human blood serum (as an example for physiological conditions). These modifications were found to protect oligonucleotides against exo- and endonucleolytic attack. The introduction of only three branches within 15mer oligonucleotide significantly retards the degradation by S1 nuclease. Also, the introduction of such a branches protect oligonucleotides from serum exonucleolyticaly degradation.

The properties of these compounds in gene inhibition were described. 17mer antisense oligonucleotides were designed, targeting K-ras point mutation on the growth of cultured human pancreatic cancer cells (PANC-1). These oligonucleotides were protected by 3`-terminal inversion (INV-oligos) against degradation by 3`-exonucleases, and one to three phosphoramidate internucleotide linkages substituted with α,ω-diamino-triethylenglycol against degradation by endonucleases. Fluorescein group was introduced at the 5`-end to visualize the cellular uptake. The oligonucleotides were synthesized, purified, and transfected into the cells by the liposome-mediated method. Cell-growth activities were estimated by MTT assay, and K-ras p21 protein synthesis was

evaluated with Western blotting. These antisense oligonucleotides were found to inhibit the expression of the mutated K-ras p21 protein in PANC-1 cells. The down-regulation increased with increasing number of branches, with a nearly complete inhibition of the p21 protein biosynthesis with an oligonucleotide containing three phosphoramidate branches.

In my first Postdoctoral position at Iowa State University, I did some research using the antisense technology to identify the regions within a mRNA that are accessible for hybridization. After that I turned to work with aptamers.

Aptamers are nucleic acids that specifically and selectively bind their target molecules. They offer an alternative to monoclonal antibodies for targeting specific proteins. These small RNA or DNA molecules can be selected by *in vitro* procedure called SELEX (Systematic Evolution of Ligands by Exponential Enrichment). We were using aptamers to design a probe to image gene expression *in vivo*. This nucleic acid probe is consisting of three regions. Aptamer, antisense and attenuator regions. In the absence of target mRNA, this probe is closed in a stem loop and the aptamer is inactive. When the mRNA hybridizes with the recognition module, the aptamer is released from the stem and binds the imaging module.

Another *long range goal* of our research is to develop effective reagents for targeted cancer therapy. The proposal focuses on the development of an aptamer-based reagent for prostate cancer. The reagent called "Drugcart" (Drug carrying aptamers for receptor targeting).

In my second Postdoctoral position at University of California, I was working on the multi-step synthesis of modified nucleosides and nucleotides. Then, I used these modified monomers to build the corresponding nucleic acid (oligonucleotide). Testing of these novel anticancer agents in biological systems (as antisense or siRNA) was also considered.

In my current position, in addition to my teaching schedule, I have around seven to nine students every semester enjoying their undergraduate research in my laboratory. We are working on developing methods for preparation of nucleic acid therapeutics called RNG. Replacement of the negatively charged phosphodiester linkages of RNA with positively charged guanidinum linkages provides the polycationic ribonucleic guanidine (RNG). The resulting compounds are designed to be incorporated into standard DNA/RNA synthesis technology. A multi-step synthetic procedure is essential for the preparation of the building blocks, including the isolation of new chemical compounds. Purification and analysis of each product is necessary to determine the chemical structure and to proceed with the next step in the synthetic procedure.

My undergraduate students and myself focus on having a research quality for publications in international journals, in addition to interest in presenting our work at several scientific meetings and conferences.

Synthesis of Sulfonamide-Ribonucleoside conjugates as potent antimicrobial, antitumor, or antiviral agents

Proposal Summary

Sulfonamides are an important class of compounds that have been reported as antibacterial, anticancer, and antiviral drugs. At least two clinically used HIV protease inhibitors possess sulfonamide moieties in their molecules, whereas a very large number of other sulfonamide derivatives are constantly being synthesized and evaluated in order to obtain compounds with less toxicity or activity against drug-resistant microbes and viruses. Nucleoside analogs have shown promising results not only as therapeutic agents, but also as potent antimicrobial agents. With deliberate chemical modifications, nucleoside analogs elicit therapeutic effects by inhibiting cancer cell growth and disrupting viral replication. Nucleoside antibiotic, e.g. A201A, with potent antibacterial activities has also been reported. A problem of growing concern is resistance to antibiotics by bacterial pathogens, which increases the demand for newly synthesized antimicrobial agents. In this proposed project, effective methods toward the synthesize of sulfonamide-ribonucleoside conjugated molecules will be investigated. The synthesized molecules will be evaluated concurrently or in a future work for their antimicrobial and anti cancer activities, and their effect will be compared to these of the individual molecules. This proposal will lay groundwork for the pursuit of external funding to develop several nucleoside HIV reverse transcriptase or HIV integrase inhibitors containing sulfonamido groups and to develop sulfonamide-nucleoside analogs as effective therapeutics for treatment of cancer.

Proposal goals and outcomes

Engagement of undergraduate students in research projects is an essential element of scholarly activities. This study will enable students to participate in undergraduate research, allowing students to explore the process of science through hands-on experience. The principle goal of this proposal is to develop student-driven research projects that increase student engagement, and stimulate interest in research; projects that develop critical thinking, problem solving, creativity, self-confidence, and enhance professional communication skills. The goals and outcomes of this proposal are summarized below:

a. Describe the scientific method and how it is used to approach the scientific problems.
b. Develop and involve undergraduate students in ongoing research projects including nucleic acids research
c. Provide students with training in advanced chemical synthesis, biological assays, computational analysis, and scientific writing skills
d. Perform multi-step synthesis reactions
e. Use and apply protective groups in synthetic methodology
f. Develop research-based special laboratory topics
g. Write an articulate description of the research work and effectively utilize proofreading, editing, and revising.
h. Perform scientific literature survey; write a research paper and clearly present research findings in the form of posters or oral conferences presentations.

i. Stimulate the critical thinking and problem solving strategies so that students become eager and more interested to pursue a graduate degree.

Students engaged in these activities will be better prepared for future independent research as they build their scientific skills, reasoning and communication abilities as well as mature confidence levels. In addition, the project reflects the faculty member's commitment to building links between research, teaching and learning. Chemistry majors and Biology majors are welcome to participate in these activities that are linked to their coursework applications, amplifying students' learning experience dramatically.

Research plan and methodology

In this proposed project, students will conduct research in the interface between chemistry and biology to create molecules capable of controlling gene expression changes in biological systems. The project will focus on developing an efficient method to chemically synthesis sulfonamide-ribonucleoside conjugates. These molecules can be used to develop nucleoside-based drugs that contain the biologically active sulfonamide groups and may therefore be proposed as an effective therapeutics for viral infections and cancer diseases. The synthetic methodology will utilize a versatile catalytic system for N-alkylation of sulfonamides with 5'-hydroxyles of various nucleosides based on a catalytic hydrogen transfer reaction that has been reported under a low catalyst loading of $[Cp^*IrCl_2]_2$ in the presence of *t*-BuOK. The faculty member will introduce this synthetic method to students, and will propose alternative solutions for possible problems. Students will then perform literature survey, collect reported protocols, and with the guidance's of the faculty research mentor will develop their synthetic schemes. Their proposals will be discussed with the research mentor before an efficient synthetic route will be recommended. Students will work on the recommended synthetic schemes to synthesize these molecules starting with commercially available reagents. Isolation, purification by column chromatography, and characterization by mass spectrometry, and nuclear magnetic resonance NMR will be performed to confirm the purity and identity through the development of the drugs. X-ray crystallography might be also applied to approve the desired structures. All the newly synthesized derivatives will be stored or concurrently tested, based on the progress of the synthesis, for their biological activities. Agar Disc-Diffusion and determination of Minimal Inhibitory Concentration (MIC) will be performed for the antibacterial tests. Students will be consistently performing literature survey, and asked to present their results at internal and national meetings.

Two undergraduate students will be recommended to participate in this project. These students are expected to complete the organic chemistry I course before they work on the project and complete or currently enrolled in the organic chemistry II course. Students are expected to be actively working in their research project and should be excited to get their work completed and submitted for publication. They should be available and commit to work during the summer 2017 and continue in the fall 2017. The faculty will be present in the lab to mentor and work with students and available at any time to provide them with guidance and advices. In addition, the faculty will be available for a daily time for individual or group meetings to discuss the background of the research project as well as the progress of the work for each student.

Professional development benefits for faculty

As a probationary faculty in the chemistry program at CSUCI, I am encouraged to focus on developing my teaching and scholarly and creative activities.
As per the Chemistry PPS, I should publish at least two articles during my probationary period, show maximum efforts to perform publishable research on a continuous basis, and make clear my personal commitment to scientific inquiry. Beside publications, I should attend and take students to present their research, as oral or poster presentations, at scientific meetings and conferences. I should also present by myself at national and international meetings. For funds to support my research in particularly over the summer 2017 in which students as well as the faculty member need support, I am applying for the CSUCI mini-grant. I will use the results and data that will be collected and supported by this grant to apply for external grants especially the Camille and Henry Dreyfus Foundation, and the NIH AREA.

The high-quality academic research requires time. I am applying for this grant to request a support for student research assistant as well as the faculty member over the summer to develop and involve undergraduate students in meaningful ongoing research projects, and to give the faculty member additional resources that will enhance his ability to be productive teacher-scholars in the discipline.

Dissemination plan

Students will be recognized and acknowledged for their efforts. Students who produced an outstanding research will be able to submit their work for publication in peer-reviewed journals, present their works at scientific conferences and meetings e.g CSUCI SAGE, CSUPERB and the 255th ACS meeting, March 2018 in the form of poster or oral presentations. In addition, outstanding students will be nominated for research awards and scholarships.

Project timeline

This project is to be conducted during the summer 2017, and students should be available to complete their project during the fall semester 2017. All participates (students and faculty mentor) are required and expected to show commitment to complete this research project by the end of the fall 2017 semester. Students will work on their synthetic protocols characterize their new compounds, and may be starting the antimicrobial tests, and analyze their data throughout the summer. The work will be completed and a manuscript will be written and planning to submit for publication during the fall. The researchers will be highly encouraged to present their results at the American Chemical Society meeting in March 2018.

Project assessment

This project was proposed so that students can accomplish the synthesis of the project title's compounds in a timely manner. The following combined assessment will be followed:
• **Final report:** Students will be required to submit final reports that present their achievements.
• **Presentation:** Students will present their findings from the project in internal (e.g SAGE) and external meetings (CSUPERB and ACS) in the form of oral or poster presentations.

• **Research paper:** Students will be required to write a research paper following specific journal guidelines based on their experimental data.

Budget

Summer stipend for faculty: $3000
The faculty member will be available and present in the lab during the summer 2017. The faculty will mentor, work with, supervise students and perform experiments to ensure the success of the project.

Student assistants: the faculty member requests that two students will work on this project. A stipend of $1500 per student is requested. This will be paid to students over the course of the project. Students will be paid up to 10 hours per week on a base of $12/hour. However, students are required to put as much hours as needed to complete the project. Students will also be asked to enroll in the independent research course CHEM 494 in fall 2017.

Supplies: $2000 of funding is being requested for purchasing chemical reagents and solvents necessary to perform the chemical syntheses, and materials including bacteria strains and cancer cell lines to perform the biological tests. This fund will be also used to perform off-campus characterization of the synthesized chemical compounds if needed.

Supplies	$2,000
Summer stipend for faculty	$3,000
Stipend for each student assistant	$1,500/student ($3000 total)

Total	$8,000

Research and Development Minigrants for 2017-2018: Review Form

Routing Step: Initial Committee Review

Application Title: Synthesis of Sulfonamide-Ribonucleoside conjugates as potent antimicrobial, antitumor, or antiviral agents

Application ID: #000060

Review Deadline: Jan 27, 2017 11:59:00 PM

***Project Goals and Outcomes:**

The proposal sets clear goals and outcomes for the project, and it explains the steps that will be taken to realize project goals.

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Rating Scale 1 (1 weakest to 11 strongest):

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***Research Plan and Methodology:**

The proposal conveys a complete and well thought-out plan for the project that describes the activities of all individuals involved in the project. If support is requested for student research assistance, the proposal must also include a description of their role in the project and how the faculty

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Rating Scale 2 (1 weakest to 11 strongest):

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***Professional Development Benefits for the Faculty:**

The proposed makes clear how the project will advance each individual applicant's or research, scholarship, creative activity, or innovation in teaching. The proposal discusses whether the applicant(s) intend to pursue external funding and identifies those external funding opportunities.

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Rating Scale 3 (1 weakest to 11 strongest):

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***Project Benefits:**

To what extent does the proposed qualify for special consideration (e.g., applicant is probationary, applicant has not had minigrant funding in the past, applicant has been especially successful in the use of past minigrant funding, project scope is particularly ambitious but realizable).

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Rating Scale 4 (1 weakest to 11 strongest):

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***Dissemination Plans:**

The level and type of dissemination is appropriate for the project, its goals, and its outcomes.

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Rating Scale 5 (1 weakest to 11 strongest):

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***Project Timeline:**

The project goals and objectives are attainable within the timeline of the proposal.

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Rating Scale 6 (1 weakest to 11 strongest):

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***Project Assessment:**

The proposal describes how the product(s) of the project will be assessed and evaluated to determine the degree of success achieved.

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Rating Scale 7 (1 weakest to 11 strongest):

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***Project Budget:**

The proposed budget is reasonable in the context of the project description, and the project costs are necessary to achieve project goals and outcomes.

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Rating Scale 8 (1 weakest to 11 strongest):

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***Other considerations:**

To what extent does the proposed qualify for special consideration (e.g., applicant is probationary, applicant has not had minigrant funding in the past, applicant has been especially successful in the use of past minigrant funding, project scope is particularly ambitious but realizable).

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Rating Scale 9 (1 weakest to 11 strongest):

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