ACS Medicinal Chemistry Letters
Cite This: ACS Med. Chem. Lett. 2019, 10, 1450−1456
Letter
pubs.acs.org/acsmedchemlett

New Negamycin-Based Potent Readthrough Derivative Effective against TGA-Type Nonsense Mutations

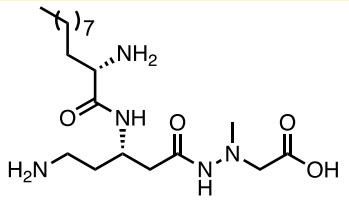
Keisuke Hamada,[†,§] Noriko Omura,[†,§] Akihiro Taguchi,[†] Alireza Baradaran-Heravi,[‡] Masaya Kotake,[†] Misaki Arai,[†] Kentaro Takayama,[†] Atsuhiko Taniguchi,[†] Michel Roberge,[‡] and Yoshio Hayashi*[,†]

[†]Department of Medicinal Chemistry, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

[‡]Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada

Supporting Information

ABSTRACT: We report a novel negamycin derivative TCP-1109 (**13x**) which serves as a potent readthrough drug candidate against nonsense-associated diseases. We previously demonstrated that TCP-112 (**7**), a nor-compound of native 3-*epi*-deoxynegmaycin, showed a higher readthrough activity than (+)-negamycin. In the present study, we performed a structure−activity relationship (SAR) study of compound **7** focused on its 3-amino group in an effort to develop a more potent readthrough compound. Introduction of a variety of natural or unnatural amino acids to the 3-amino group gave us the more potent derivative **13x** which has about four times higher readthrough activity than **7** in a cell-based assay using a premature termination codon of TGA derived from Duchenne muscular dystrophy. The activity was dose-dependent and relatively selective for TGA. However, the activities for TAG and TAA were also higher than those of (+)-negamycin and **7**. Moreover, compound **13x** showed significant cell-based readthrough activity for several nonsense mutations derived from other nonsense-associated diseases. It is suggested that **13x** has the potential to be a readthrough drug useful for the treatment of many kinds of nonsense-associated diseases.



Novel negamycin derivative TCP-1109 (**13x**)

- showed twice the readthrough activity value compared to G418
- has the potential to be a therapeutic candidate to treat a variety of TGA-type nonsense mutation-mediated diseases

KEYWORDS: *Duchenne muscular dystrophy, leucyl-3-epi-deoxynegamycin, (+)-negamycin, readthrough, premature termination codon, nonsense mutational disease*

Readthrough strategy has attracted attention recently for treatment of hereditary diseases mediated by nonsense mutations by introducing an amino acid at the premature termination codon (PTC), producing a functional full-length protein.[1] Aminoglycoside antibiotics such as gentamicin (the mixture of gentamicin C1, C1a, C2, and C2a, **1**)[2] and Geneticin (G418, **2**)[3] are well-known readthrough compounds, although G418 is known to exhibit very high toxicity in vivo.[4] It is known that aminoglycosides generally exhibit antimicrobial activity at high concentrations by inhibiting ribosomal protein synthesis. At low concentrations, however, they induce reading mistakes during translation by binding to 16S rRNA of the 30S ribosome in prokaryotic cells and 18S rRNA of the 40S ribosome in eukaryotic cells.[5,6] From a medical viewpoint in the treatment of genetic diseases, gentamicin was administered in 1990s to *mdx* mice, a mouse model for Duchenne muscular dystrophy (DMD) with a nonsense mutation of TAA in the dystrophin gene. It was reported that gentamicin induces reexpression of about 10% of the full-length form of dystrophin.[7] Long-term treatment with aminoglycosides however is known to cause serious adverse effects such as ototoxicity and nephrotoxicity[8,9] and emergence of drug-resistant bacteria.[10] Therefore, the development of new compounds with potent readthrough activity but no serious toxicity and antimicrobial activity is needed.

In recent publications, NB54,[11] NB74,[12] and NB84[12] were reported as new synthetic aminoglycosides with reduced ototoxicity. Ataluren (**3**), which is a small oxadiazole derivative discovered by chemical library screening, was also reported as a readthrough candidate in 2007.[13] Recently, ataluren was conditionally approved as a new drug in Europe, Israel, and South Korea for the treatment of DMD and investigation of the details of pharmacokinetic analysis of ataluren in Japanese and Caucasian is progressing.[14]

(+)-Negamycin (**4**),[15] a dipeptide-like hydrazide antibiotic, which was isolated from *Streptomyces purpeofuscus* in 1970, shows potent antimicrobial activity, particularly against Gram-negative bacteria. In 2003, Arakawa et al. reported that (+)-negamycin restored dystrophin expression in *mdx* mice.[16]

Received: June 18, 2019
Accepted: September 23, 2019
Published: September 23, 2019

DOI: 10.1021/acsmedchemlett.9b00273
ACS Med. Chem. Lett. 2019, 10, 1450−1456

It was also reported that (+)-negamycin has low toxicity: its lethal dose 50% (LD_{50}) is one-tenth that of gentamicin.[17] From these studies, (+)-negamycin appears to be a preferable alternative to aminoglycosides in the development of therapeutic agents for nonsense-associated diseases. Moreover, in our previous study, we found that chemically synthesized native 3-*epi*-deoxynegamycin (**5**) and leucyl-3-*epi*-deoxynegamycin (**6**), both isolated from *Streptomyces goshikiensis*, show higher readthrough activity than (+)-negamycin.[18] Interestingly, it has also been reported that these two native negamycin analogues lack antimicrobial activity, and this was recently reconfirmed in our evaluation of the synthetic derivatives.[18] Therefore, it is demonstrated that readthrough and antimicrobial activities can be separated in the *epi*-deoxynegamycin skeleton. Encouraged by this interesting result, a structure–activity relationship (SAR) study of *epi*-deoxynegamycin **5** focused on the length of backbone obtained a derivative TCP-112 (**7**) which is a derivative of **5**, but one carbon shorter.[19] This compound **7** showed a more potent readthrough activity than **5**. Moreover, esterification of the carboxyl group in **7**,

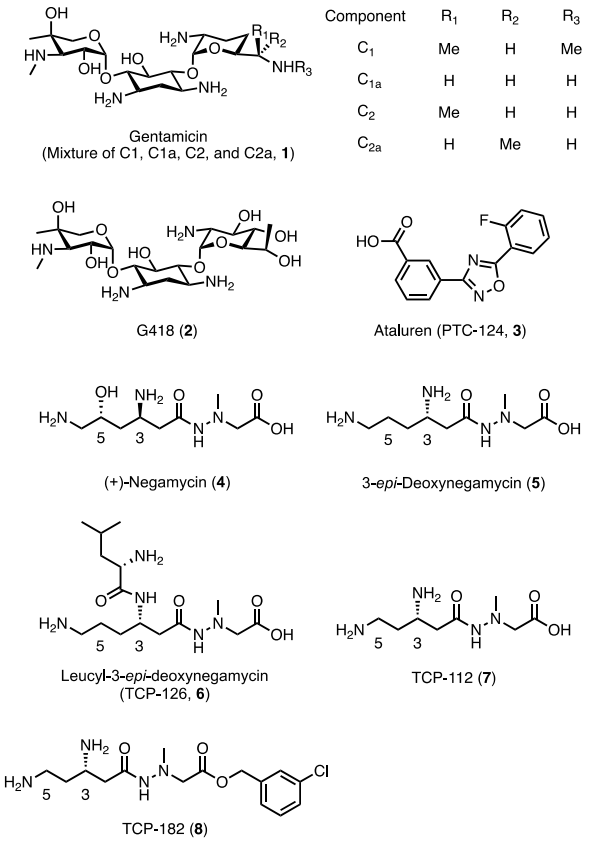
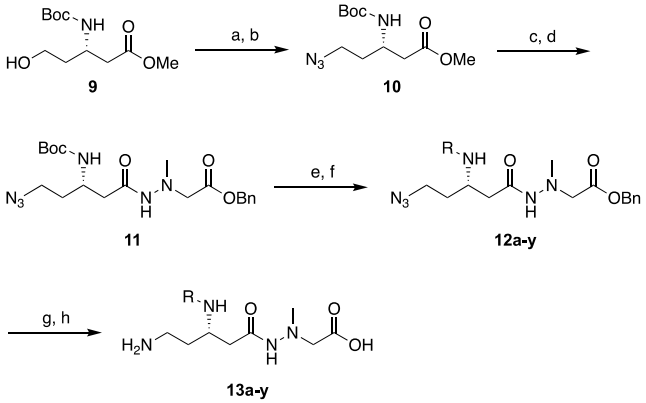


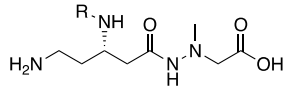
Figure 1. Structure of aminoglycosides, ataluren, natural (+)-negamycin, and its analogues.

Scheme 1. Synthesis of Derivatives 13a–y[a]



[a]Reagents and conditions: (a) MsCl, Et_3N, CH_2Cl_2, RT, overnight, 90%. (b) NaN_3, DMF, RT, overnight, 91%. (c) KOH, MeOH/H_2O, 5 h. (d) $H_2NN(Me)CH_2CO_2Bn$, EDC·HCl, HOBt·H_2O, Et_3N, DMF, RT, overnight, 81% (2 steps). (e) 4 M HCl/dioxane, RT, 1 h. (f) Protected amino acid or 4-methylpentanoic acid, EDC·HCl, HOBt·H_2O, Et_3N, DMF, RT, overnight, 49–98% (2 steps). For **13d**, (g) H_2, Pd/C, MeOH, RT, 1 h, then RP-HPLC, 27% (2 steps). For **13a–c** and **13e–y**, (h) H_2, Pd/C, MeOH, RT, 1 h, then, 4 M HCl/dioxane, RT, 1 h, then RP-HPLC, 7–64% (3 steps).

Table 1. Cell-Based Readthrough Activity of Derivative 13a–y



13

compd	R	readthrough activity[ab]
G418 (**2**)	na[c]	7.99 ± 0.14
(+)-negamycin (**4**)	na[c]	2.25 ± 0.17
TCP-112 (**7**)	H	4.26 ± 0.06
13a	L-Leu	4.94 ± 0.18
13b	D-Leu	1.46 ± 0.09
13c	L-N(Me)-Leu	2.45 ± 0.22
13d	4-methylpentanoyl	2.82 ± 0.65
13e	Gly	2.74 ± 0.13
13f	L-Ala	2.40 ± 0.05
13g	L-Ser	1.48 ± 0.11
13h	L-Dap	3.02 ± 0.92
13i	L-Dab	3.25 ± 0.14
13j	L-Orn	2.96 ± 0.40
13k	L-Lys	2.49 ± 0.16
13l	L-Asp	1.96 ± 0.20
13m	L-Glu	1.80 ± 0.18
13n	L-Asn	2.21 ± 0.13
13o	L-Gln	1.62 ± 0.12
13p	L-Tyr	2.59 ± 0.13
13q	L-Phe	2.99 ± 0.35
13r	L-Val	2.00 ± 0.09
13s	L-Ile	1.83 ± 0.08
13t	L-Cha	5.87 ± 0.39
13u	L-Nle	4.21 ± 0.65
13v	L-Ahp (**2**)	3.40 ± 0.31
13w	L-Anon (**2**)	7.97 ± 0.47
13x (TCP-1109)	L-Aund (**2**)	15.96 ± 1.04
13y	L-α-aminotridecanoic acid	nd[d]

[a]In vitro readthrough activity in COS-7 cells against TGA-type PTC originally derived from nonsense mutation seen in DMD. A dual reporter plasmid encoding β-galactosidase and luciferase genes that were connected with a TGA-containing nucleotide sequence was used. The values are ratios relative to a control (D-MEM). Compounds were evaluated at a concentration of 200 μM. [b]Cell-based readthrough activity (ratio) relative to TCP-112 (**7**) (= 4.26) in COS-7 cells.[19] Compounds were evaluated at a concentration of 200 μM. Values are the mean ± SD (n = 3) [c]na: Not applicable. [d]nd: Not detected.

DOI: 10.1021/acsmedchemlett.9b00273
ACS Med. Chem. Lett. 2019, 10, 1450–1456

leading to form a prodrug structure, produced a more potent derivative, TCP-182 (**8**) with a *m*-chlorobenzyl ester. Incubation of **8** with porcine esterase in vitro produced the parent derivative **7** quantitatively (Figure 1).[19]

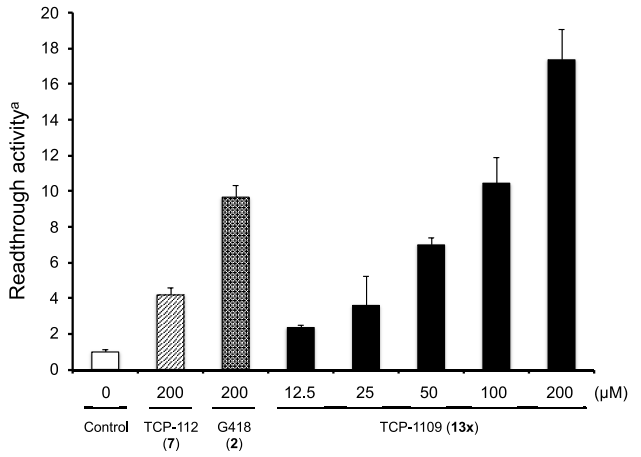


Figure 2. Readthrough activity of TCP-1109 (**13x**) against TGA sequence. Cell-based readthrough activity (ratio) relative to control (D-MEM) in COS-7 cells. Values are expressed as the mean ± SD (n = 3).

Table 2. Cell-Based Readthrough Activity of Derivative 13x to Several Nonsense Mutations

compd	readthrough activity[a]		
	-TGA-	-TAG-	-TAA-
G418 (**2**)	7.43 ± 0.22	7.86 ± 0.21	4.78 ± 0.14
(+)-negamycin (**4**)	1.47 ± 0.15	1.17 ± 0.31	1.13 ± 0.01
TCP-126 (**6**)	2.30 ± 0.05	1.30 ± 0.06	1.11 ± 0.05
TCP-112 (**7**)	3.80 ± 0.69	1.48 ± 0.07	1.20 ± 0.18
TCP-1109 (**13x**)	18.3 ± 0.26	4.39 ± 0.04	2.24 ± 0.41

[a]Cell-based readthrough activity (ratio) relative to control (D-MEM) in COS-7 cells; compounds are evaluated at a concentration of 200 μM. Values are the mean ± SD (n = 3).

In an alternative SAR study based on leucyl-3-*epi*-deoxynegamycin (**6**), we synthesized six derivatives with substitution at the Leu residue, but failed to increase the readthrough activity.[20] Consequently, in the present study, we focused on a more potent nor-compound of **5**, i.e., **7**, and modified its 3-amino position with a variety of amino acid residues in an attempt to develop more potent readthrough compounds. The synthesized derivatives were evaluated in an in vitro readthrough assay using COS-7 cells.[18] This led to the discovery of a novel negamycin derivative TCP-1109 (**13x**) with a highly potent readthrough activity. We also constructed novel plasmids and performed cell-based evaluation for readthrough efficiency of negamycin derivatives to ascertain their versatile activity with respect to other nucleotide sequences involving nonsense mutations derived from non-sense-associated diseases other than DMD.

The chemical structures of the new derivatives are shown in Table 1, and the syntheses of derivatives **13a**−**y** are depicted in Scheme 1. The alcohol **9**[21] was mesylated, and this was followed by treatment with NaN_3 to obtain the azide derivative **10**. After hydrolysis of the methyl ester in **10** with potassium hydroxide, the resulting carboxylic acid was coupled with a hydrazine unit using a 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide (EDC)-1-hydroxybenzotriazole (HOBt) method to give the hydrazide **11**. The Boc group of the hydrazide **11** was deprotected by treatment with 4 M HCl/dioxane, and this was followed by coupling with various amino acid derivatives and 4-methyl pentanoic acid that corresponds to deamino-Leu to obtain derivatives **12a**−**y**. Reduction of the azide group and deprotection of the benzyl ester in **12a**−**y** were performed by Pd/C catalyzed hydrogenolysis, followed by the deprotection of the Boc group by treatment with 4 M HCl/dioxane. HPLC purification of the crude product afforded 25 new derivatives **13a**−**y**.

To understand the effect on the readthrough activity of introducing acyl units into the amino group at the 3-position of derivative **7**, we investigated the in vitro readthrough activity of the compounds using a previously described procedure in a cell-based reporter assay against TGA-type PTC originally derived from the nonsense mutation found in DMD.[18] The reporter consisted of a dual reporter plasmid encoding β-galactosidase and luciferase genes that are connected with a TGA-containing nucleotide sequence used as a PTC. As shown in Table 1, the introduction of L-Leu (**13a**) which is present in the native analogue **6**, slightly increased the readthrough activity with a value of 4.94 ± 0.18 compared to the starting derivative **7** (4.26 ± 0.06), while the introduction of D-Leu (**13b**) significantly decreased the activity (1.46 ± 0.09). This result is consistent with that observed in the D-Leu substitution performed with the native analogue **6**.[20] Therefore, the L-stereochemistry originating from the native structure is crucial to the activity, and this is accurately reflected in the following modification of derivative **7**. The *N*-methylation (**13c**) and removal of the α-amino group (**13d**) at L-Leu decreased the activity about 2-fold (2.45 ± 0.22 and 2.82 ± 0.65, respectively) in comparison to derivative **7**, suggesting that the existing unmodified α-amino group at L-Leu is also an important factor necessary for potent activity.

In order to investigate the effect of the side chain of L-Leu in derivative **13a**, several derivatives in which L-Leu is replaced with various kinds of amino acids were synthesized and their readthrough activity was evaluated. However, most derivatives (**13e**−**s**) in which proteinogenic amino acids, Dap, Dab, and Orn were incorporated showed a reduced readthrough activity ranging from 1.48 to 3.25. In particular, those with hydrophilic amino acid Ser (**13g**), Asn (**13n**), or Gln (**13o**), acidic amino acid Asp (**13l**) or Glu (**13m**), or β-branched amino acid Val (**13r**) or Ile (**13s**) decreased the activity more than 2-fold compared to the starting derivative **7**. Substitutions with an aromatic amino acid Phe (**13q**) or a basic amino acids Dap (**13h**), Dab (**13i**), or Orn (**13j**) were relatively tolerated, retaining about 70% of the activity of derivative **7**.

However, improvement in the activity was observed when an amino acid with a longer alkyl side chain was introduced, with the exception of Ahp (L-α-aminoheptanoic acid, **13v**). The derivative with norleucine (Nle, **13u**) showed a similar activity (4.21 ± 0.65) to that of **7** and **13t**, with Cha (L-cyclohexylalanine) showing a higher readthrough activity (5.87 ± 0.39) than that of **7**. Moreover, derivatives with an even longer normal alkyl chain enhanced the readthrough activity approximately in proportion to the length of the carbon chain. Derivatives with Anon (L-α-aminononoic acid, **13w**) and Aund (L-α-aminoundecanoic acid, **13x**, TCP-1109) increased the activity by about 2- and 4-fold that of derivative **7** with readthrough activities of 7.97 ± 0.47 and 15.96 ± 1.04, respectively. The activity of **13x**, which has an *n*-nonyl side

DOI: 10.1021/acsmedchemlett.9b00273
ACS Med. Chem. Lett. 2019, 10, 1450−1456

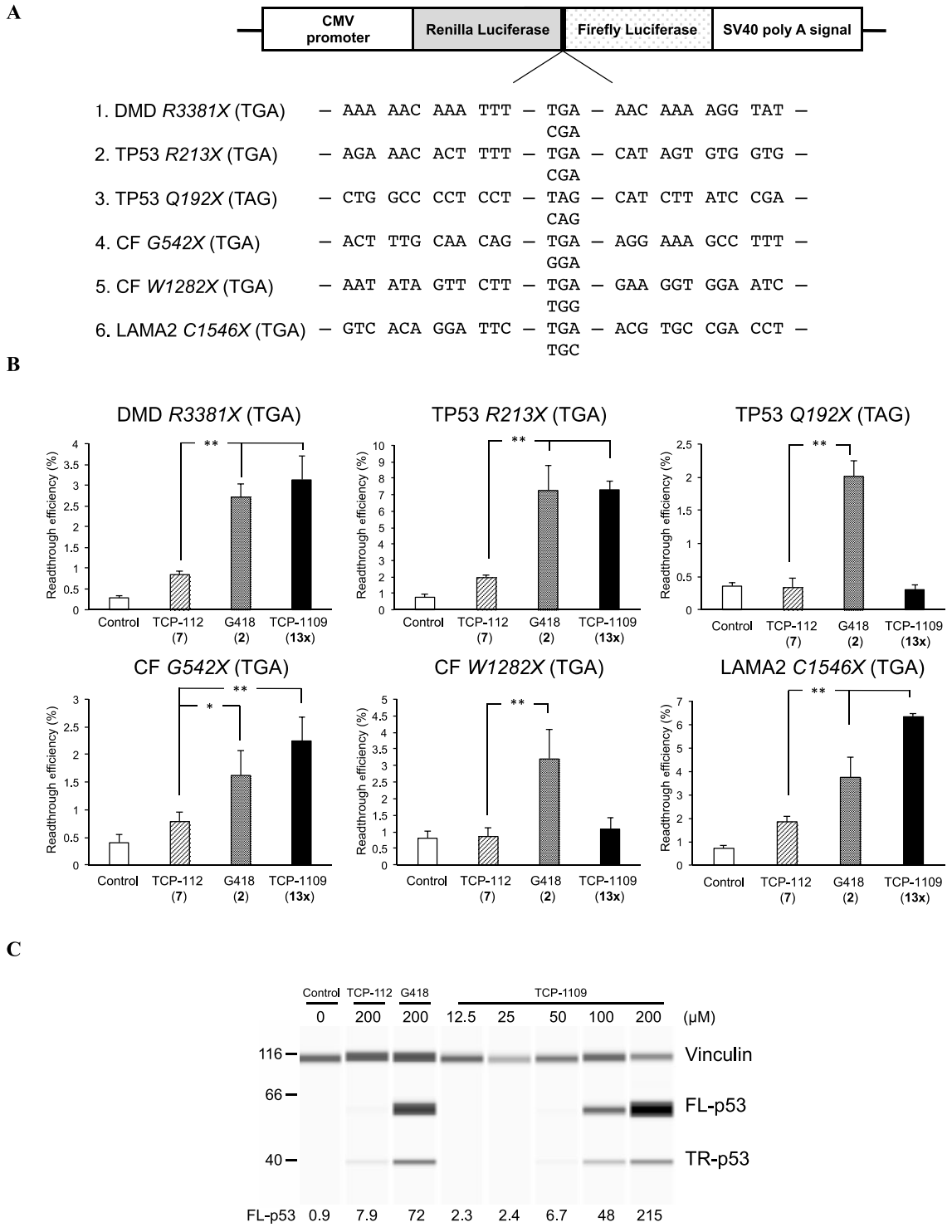


Figure 3. (A) Structure of the dual luciferase reporter plasmid containing several nonsense mutational diseases sequence. The sequence below each PTC containing plasmid means the corresponding wild-type plasmid. (B) In vitro readthrough efficiency against several nonsense mutation sequences relative to control (D-MEM) in COS-7 cells; compounds were evaluated at a concentration of 200 μM. Values are expressed as the mean ± SD (n = 3). **p < 0.01 and *p < 0.05. (C) PTC readthrough at endogenous p53 *R213X* in HDQ-P1 human breast carcinoma cells. HDQ-P1 cells were exposed to different concentrations of TCP-112, TCP-1109, or G418 for 72 h, and full-length p53 (FL-p53) and truncated p53 (TR-p53) were determined by automated capillary electrophoresis western analysis. Vinculin was used as a protein loading control. The results are displayed as pseudo blots. FL-p53 peak intensity (p53 readthrough) is displayed under each lane, normalized to vinculin.

chain, is 10-fold that of (+)-negamycin (**3**) and 2-fold that of the most potent native readthrough aminoglycoside G418. In addition, compound **13x** exhibited a dose-dependent readthrough activity in the same cell-based assay at concentrations ranging from 12.5 to 200 μM (Figure 2) against TGA-type PTC. On the other hand, the derivative **13y**, with an even longer alkyl chain (undecyl group), i.e., L-α-aminotridecanoic acid, showed cytotoxicity (the IC_{50} value on the COS-7 cell

DOI: 10.1021/acsmedchemlett.9b00273
ACS Med. Chem. Lett. 2019, 10, 1450−1456

line was determined to be 68.2 ± 2.00 μM by WST-1 assay), hindering evaluation of the readthrough activity (data not shown). From these results, it could be seen that introduction of amino acids having a flexible and hydrophobic long alkyl chain is well-tolerated, increasing the readthrough activity at the 3-position of derivative **7** (TCP-112).

To understand the details of the activity of **13x**, we evaluated its readthrough activity to other PTCs in the cell-based assay using the same dual reporter plasmid but with a different TAG or TAA-type PTC.[18] As shown in Table 2, G418 has a relatively equivalent readthrough activity level to the three PTCs in the order TAG = TGA > TAA (7.86, 7.43 and 4.78, respectively). On the other hand, as in our previous report,[18] (+)-negamycin (**4**) showed moderate activity only to TGA, and derivatives **6** and **7** showed increased activity, particularly to TGA. Hence, the PTC preference of these negamycin derivatives is in the order of TGA ≫ TAG ≥ TAA. Derivative **13x** also exhibited a similar profile with the best selectivity to TGA; however, **13x** has a much stronger effect on the other PTCs TAG and TAA than any other negamycin derivatives with a readthrough activity of 4.39 and 2.24, respectively. This result indicates that **13x** is also relatively effective to TAG and TAA, although its activities are still weaker than that of G418.

To date we evaluated the readthrough activity as the ratio against the control with no compound. In the present study, in order to enable the calculation of the percent of readthrough efficiency, we constructed 12 new plasmids consisting of a dual-reporter encoding renilla and firefly luciferase genes with or without PTCs as shown in Figure 3A. Two types of PTCs, TGA and TAG, were used, and peripheral nucleotide sequences were selected from several typical nonsense mutations, i.e. *R3381X*[22] observed at dystrophin (*DMD* gene product) in DMD, *R213X* and *Q192X* observed at p53,[23] (*TP53* gene product) in cancer, *G542X* and *W1282X* observed at the cystic fibrosis transmembrane conductance regulator[24,25] (*CFTR* gene product) in cystic fibrosis (CF) and *C1546X* observed at laminin α2 chain (*LAMA2* gene product) in congenital muscular dystrophy type 1A (MDC1A).[26,27] Using these plasmids, we evaluated the cell-based readthrough activity of representative derivatives including derivative **7**, the most potent compound **13x** obtained in the present SAR study and G418 as a positive control. The readthrough efficiency (%) of each compound at 200 μM was calculated by dividing the readthrough activity (firefly/renilla ratio) obtained in the use of each plasmid with PTC by the firefly/renilla expression ratio obtained in the absence of PTC which was defined as 100% efficient. The results are shown in Figure 3B. In comparison to the control with no compound, G418 generally showed relatively good readthrough efficiency ranging from 1.62 to 7.25% with a similar preference between two PTCs (TGA and TAG). On the other hand, derivative **7** was only effective with two TGAs, *R213X* (*TP53*) and *C1546X* (*LAMA2*), with a moderate activity of 1.95 and 1.85%, respectively. Derivative **13x** showed readthrough efficiency to four TGAs of which three PTCs, *R3381X* (*DMD*), *R213X* (*TP53*), and *G542X* (*CFTR*), showed a similar efficiency (2.25−7.30%), and *C1546X* (*LAMA2*) revealed a stronger efficiency (6.34%) in comparison to G418.

In the case of TAG derived from *Q192X* (*TP53*), **13x** does not induce readthrough at all. Bidou et al. demonstrated that the possibility of readthrough is not only generally dependent on the kind of PTC, i.e., hierarchically in the order of TGA > TAG > TAA, but is also influenced by nucleotide sequences around PTCs.[28] Negamycin and **13x** showed a similar PTC preference of TGA > TAG. However, against two TGA mutations derived from cystic fibrosis, derivative **13x** was only active with *G542X* with an efficiency of 2.25% and was inactive with *W1282X*. This difference in the readthrough efficiency of derivative **13x** indicates that readthrough efficiency is influenced by the nucleotide sequence around PTC to a great extent. Further investigation is needed to elucidate the precise influence of nucleotides surrounding the PTC on the activity of negamycin derivatives, but it was found that derivative **13x** is effective in several nonsense mutations having TGA with a potent readthrough efficiency comparable to that of G418. This suggested that **13x** has a potential to be a readthrough drug useful for the treatment of many nonsense-associated diseases. To justify the potential to be a readthrough drug, we tested **13x** in one cellular model where readthrough of an endogenous mutated gene is measured. We chose HDQ-P1 human breast carcinoma cells containing the endogenous p53 *R213X* (TGA) homozygous mutation. HDQ-P1 cells were exposed to different concentrations of TCP-112, TCP-1109, or G418 for 72 h, and full-length p53 (FL-p53), the readthrough product, and truncated p53 (TR-p53) were determined by automated capillary electrophoresis western analysis.[29] As shown in Figure 3C, **13x** (200 μM) showed three times higher readthrough activity than G418 (200 μM) did with no visible signs of cytotoxicity. This result clearly showed that **13x** has also strong activity against an endogenous gene.

In summary, to develop a potent negamycin-based readthrough compound, a SAR study of negamycin derivative **7** (TCP-112) was performed, focused on its 3-amino position using a TGA-based in vitro readthrough assay, and a highly potent readthrough derivative **13x** (TCP-1109) was discovered. **13x** has twice the activity value of G418 and exhibits dose-dependent readthrough activity in vitro in a cell-based reporter assay against TGA-type PTC originally derived from the nonsense mutation found in DMD. Further cell-based evaluation that can calculate the readthrough efficiency demonstrated that **13x** shows significant readthrough efficiency against several other nonsense sequences including TGA derived from *DMD*, *TP53*, *CFTR*, and *LAMA2* with an efficiency comparable to G418. Moreover, **13x** showed strong readthrough activity in HDQ-P1 cells containing the endogenous p53 *R213X* (TGA) mutation. These results suggested that **13x** (TCP-1109) has the potential to be a therapeutic candidate to treat a variety of TGA-type nonsense mutation mediated hereditary diseases.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.9b00273.

Synthesis procedures, characterization of derivatives, biological assay protocols, and NMR data (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Telephone/fax: +81 42 676 3275. E-mail: yhayashi@toyaku.ac.jp.

ORCID

Yoshio Hayashi: 0000-0002-7010-6914

DOI: 10.1021/acsmedchemlett.9b00273
ACS Med. Chem. Lett. 2019, 10, 1450−1456

Author Contributions

[§]K.H. and N.O. contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Japan Society for the Promotion of Science (JSPS), KAKENHI including a Grant-in-Aid for JSPS Research Fellow 16J08454, a Grant-in-Aid for Scientific Research (B) 23390029, 19H03356, a Grant-in-Aid for Young Scientists (B) 25860092, a Grant-in-Aid for Research Activity Start-up 25893258, and a Grant-in-Aid for Scientific Research on Innovative Areas "Chemical Biology of Natural Products" from the Ministry of Education, Culture, Sports, Science and Technology, and Platform for Drug Discovery, Informatics and Structural Life Science, and Intramural Research Grant (29-4) for Neurological and Psychiatric Disorders of NCNP. MEXT-supported Program for the Strategic Research Foundation at Private Universities. Support was also provided by the Canadian Cancer Society (Grant #704700) and the Michael Smith Foundation for Health Research.

■ ABBREVIATIONS

PTCs, premature termination codons; DMD, Duchenne muscular dystrophy; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; SAR, structure−activity relationship; EDC, 1-ethyl-3-(3-(dimethylamino)-propyl)carbodiimide; HOBt, 1-hydroxy-benzotriazole; Et_3N, triethylamine; DMF, *N*,*N*-dimethylformamide; RP-HPLC, reversed-phase high performance liquid chromatography; Boc, *tert*-butoxycarbonyl; D-MEM, Dulbecco's modified Eagle's medium; Dap, 2,3-diaminopropionic acid; Dab, 2,4-diaminobutyric acid; Orn, ornithine; CHA, cyclohexylalanine; Nle, norleucine; Ahp, α-aminoheptanoic acid; Anon, α-aminononoic acid; Aund, α-aminoundecanoic acid

■ REFERENCES

(1) Howard, M.; Frizzell, R. A.; Bedwell, D. M. Aminoglycoside antibiotics restore CFTR function by overcoming premature stop mutations. *Nat. Med.* **1996**, *2*, 467−469.

(2) Martin, R.; Mogg, A. E.; Heywood, L. A.; Nitschke, L.; Burke, J. F. Aminoglycoside suppression at UAG, UAA and UGA codons in Escherichia coli and human tissue culture cells. *Mol. Gen. Genet.* **1989**, *217*, 411−418.

(3) Keeling, K. M.; Bedwell, D. M. Pharmacological suppression of premature stop mutations that cause genetic diseases. *Curr. Pharmacogenomics* **2005**, *3*, 259−269.

(4) Floquet, C.; Rousset, J. P.; Bidou, L. Readthrough of premature termination codons in the adenomatous polyposis coli gene restores its biological activity in human cancer cells. *PLoS One* **2011**, *6*, No. e24125.

(5) Fourmy, D.; Recht, M. I.; Blanchard, S. C.; Puglisi, J. D. Structure of the site of Escherichia coli 16S ribosomal RNA complexed with an aminoglycoside antibiotic. *Science* **1996**, *274*, 1367−1371.

(6) Prokhorova, I.; Altman, R. B.; Djumagulov, M.; Shrestha, J. P.; Urzhumtsev, A.; Ferguson, A.; Chang, C. T.; Yusupov, M.; Blanchard, S. C.; Yusupova, G. Aminoglycoside interactions and impacts on the eukaryotic ribosome. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, E10899−E10908.

(7) Wagner, K. R.; Hamed, S.; Hadley, D. W.; Gropman, A. L.; Burstein, A. H.; Escolar, D. M.; Hoffman, E. P.; Fischbeck, K. H. Gentamicin treatment of Duchenne and becker muscular dystrophy due to nonsense mutation. *Ann. Neurol.* **2001**, *49*, 706−711.

(8) Mingeot-Leclercq, M. P.; Tulkens, P. M. Aminoglycosides: nephrotoxicity. *Antimicrob. Agents Chemother.* **1999**, *43*, 1003−1012.

(9) Hutchin, T.; Cortopassi, G. Proposed molecular and cellular mechanism for aminoglycoside ototoxicity. *Antimicrob. Agents Chemother.* **1994**, *38*, 2517−2520.

(10) Garneau-Tsodikova, S.; Labby, K. J. Mechanisms of resistance to aminoglycoside Antibiotics: overview and perspectives. *MedChemComm* **2016**, *7*, 11−27.

(11) Nudelman, I.; Rebibo-Sabbah, A.; Cherniavsky, M.; Belakhov, V.; Hainrichson, M.; Chen, F.; Schacht, F.; Pilch, D. S.; Ben-Yosef, T.; Baasov, T. Development of novel aminoglycoside (NB54) with reduced toxicity and enhanced suppression of disease-causing premature stop mutations. *J. Med. Chem.* **2009**, *52*, 2836−2845.

(12) Nudelman, I.; Smolkin, B.; Hainrichson, M.; Balekhov, V.; Baasov, T. Repairing faulty genes by aminoglycosides: Development of new derivatives of geneticin (G418) with enhanced suppression of diseases-causing nonsense mutations. *Bioorg. Med. Chem.* **2010**, *18*, 3735−3746.

(13) Welch, E. M.; Barton, E. R.; Zhuo, J.; Tomizawa, Y.; Friesen, W. J.; Trifillis, P.; Paushkin, S.; Patel, M.; Trotta, C. R.; Hwang, S.; Wilde, R. G.; Karp, G.; Takasugi, J.; Chen, G.; Jones, S.; Ren, H.; Moon, Y. C.; Corson, D.; Turpoff, A. A.; Campbell, J. A.; Conn, M. M.; Khan, A.; Almstead, N. G.; Hedrick, J.; Mollin, A.; Risher, N.; Weetall, M.; Yeh, S.; Branstrom, A. A.; Colacino, J. M.; Babiak, J.; Ju, W. D.; Hirawat, S.; Northcutt, V. J.; Miller, L. L.; Spatrick, P.; He, F.; Kawana, M.; Feng, H.; Jacobson, A.; Peltz, S. W.; Sweeney, H. L. PTC124 targets genetic disorders caused by nonsense mutations. *Nature* **2007**, *447*, 87−91.

(14) Kong, R.; Laskin, O. L.; Kaushik, D.; Jin, F.; Ma, J.; McIntosh, J.; Souza, M.; Almstead, N. Ataluren pharmacokinetics in healthy Japanese and Caucasian subjects. *Clin. Pharmacol. Drug Dev.* **2019**, *8*, 172−178.

(15) Hamada, H.; Tadeuchi, T.; Kondo, S.; Ikeda, Y.; Naganawa, H.; Maeda, K.; Okami, Y.; Umezawa, H. A new antibiotic, negamycin. *J. Antibiot.* **1970**, *23*, 170−171.

(16) Arakawa, M.; Shiozuka, M.; Nakayama, Y.; Hara, T.; Hamada, M.; Kondo, S.; Ikeda, D.; Takahashi, Y.; Nonomura, Y.; Sheykholeslami, K.; Kondo, K.; Kaga, K.; Kitamura, T.; Suzuki-Miyagoe, Y.; Takeda, S.; Matsuda, R. Negamycin restores dystrophin expression in skeletal and cardiac muscles of *mdx* mice. *J. Biochem.* **2003**, *134*, 751−758.

(17) Hamada, M.; Takauchi, T.; Kondo, S.; Ikeda, Y.; Naganawa, H. A new antibiotic, negamycin. *J. Antibiot.* **1970**, *23*, 170−171.

(18) Taguchi, A.; Hamada, K.; Kotake, M.; Shiozuka, M.; Nakaminami, H.; Pillaiyar, T.; Takayama, K.; Yakushiji, F.; Noguchi, N.; Usui, T.; Matsuda, R.; Hayashi, Y. Discovery of natural products possessing selective eukaryotic readthrough activity: 3-*epi*-deoxynegamycin and its leucine adduct. *ChemMedChem* **2014**, *9*, 2233−2237.

(19) Hamada, K.; Taguchi, A.; Kotake, M.; Aita, S.; Murakami, S.; Takayama, K.; Yakushiji, F.; Hayashi, Y. Structure-activity relationship studies of 3-*epi*-deoxynegamycin derivatives as potent readthrough drug candidates. *ACS Med. Chem. Lett.* **2015**, *6*, 689−694.

(20) Taguchi, A.; Hamada, K.; Shiozuka, M.; Kobayashi, M.; Murakami, S.; Takayama, K.; Taniguchi, A.; Usui, T.; Matsuda, R.; Hayashi, Y. Structure-activity relationship study of leucyl-3-*epi*-deoxynegamycin for potent premature termination codon read-through. *ACS Med. Chem. Lett.* **2017**, *8*, 1060−1065.

(21) Lesma, G.; Danieli, B.; Sacchetti, A.; Silvani, A. An efficient enantioselective approach to cyclic β-amino acid derivatives via olefin metathesis reactions. *J. Org. Chem.* **2006**, *71*, 3317−3320.

(22) Tuffery-Giraud, S.; Chambert, S.; Demaille, J.; Claustres, M. Point mutations in the dystrophin gene: Evidence for frequent use of cryptic splice sites as a result of splicing defects. *Hum. Mutat.* **1999**, *14*, 359−368.

(23) Floquet, C.; Deforges, J.; Rousset, J. P.; Bidou, L. Rescue of non-sense mutated p53 tumor suppressor gene by aminoglycosides. *Nucleic Acids Res.* **2011**, *39*, 3350−3362.

DOI: 10.1021/acsmedchemlett.9b00273
ACS Med. Chem. Lett. 2019, 10, 1450−1456

(24) Cystic fibrosis genotype-phenotype consortium.. Cystic fibrosis genotype-phenotype consortium. Correlation between genotype and phenotype in patients with cystic fibrosis. *N. Engl. J. Med.* **1993**, *329*, 1308−1313.
(25) Shoshani, T.; Kerem, E.; Szeinberg, A.; Augarten, A.; Yahav, Y.; Cohen, D.; Rivlin, J.; Tal, A.; Kerem, B. Similar levels of mRNA from the W1282X and the delta F508 cystic fibrosis alleles, in nasal epithelial cells. *J. Clin. Invest.* **1994**, *93*, 1502−1507.
(26) Allamand, V.; Guicheney, P. Merosin-deficient congenital muscular dystrophy, autosomal recessive (MDC1A, MIM#156225, LAMA2 gene coding for alpha2 chain of laminin). *Eur. J. Hum. Genet.* **2002**, *10*, 91−94.
(27) Helbling-Leclerc, A.; Zhang, X.; Topaloglu, H.; Cruaud, C.; Tesson, F.; Weissenbach, J.; Tome, F. M.; Schwartz, K.; Fardeau, M.; Tryggvason, K.; et al. Mutations in the laminin alpha 2-chain gene (LAMA2) cause merosin-deficient congenital muscular dystrophy. *Nat. Genet.* **1995**, *11*, 216−218.
(28) Floquet, C.; Hatin, I.; Rousset, J.-P.; Bidou, L. Statistical analysis of readthrough levels for nonsense mutations in mammalian cells reveals a major determinant of response to gentamicin. *PLoS Genet.* **2012**, *8*, No. e1002608.
(29) Baradaran-Heravi, A.; Balgi, A. D.; Zimmerman, C.; Choi, K.; Shidmoosavee, F. S.; Tan, J. S.; Bergeaud, C.; Krause, A.; Flibotte, S.; Shimizu, Y.; Anderson, H. J.; Jan, E.; Pfeifer, T.; Jaquith, J. B.; Roberge, M. Novel small molecules potentiate premature termination codon readthrough by aminoglycosides. *Nucleic Acids Res.* **2016**, *44*, 6583−6598.

DOI: 10.1021/acsmedchemlett.9b00273
ACS Med. Chem. Lett. 2019, 10, 1450−1456