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A Convenient Synthetic Pathway for Multivalent Assembly of Aminoglycoside Antibiotics Starting from Amikacin

Hidehiko Tanaka,[a] Yoshihiro Nishida,[a] Yousuke Furuta[b] and Kazukiyo Kobayashi[a,*]

[a]*Department of Molecular Design and Engineering, Graduate School of Engineering, Nagoya University, Chikusa-ku, Nagoya 464-8603, Japan*

[b]*Toyama Kagaku Kogyo Co. Ltd, Toyama City, Toyama 930-8508, Japan*

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Abstract—Vinylpolymers carrying a kanamycin cluster at the side chain were prepared via regioselective *N*-acylation of amikacin with *N*-succinimidyl *p*-vinylbenzoate, followed by radical homo- and co-polymerization with acrylamide. Two independent biological assays disclosed that the polyvalent kanamycin models showed neither antibacterial activity nor inhibitory activity againt rRNA-based protein synthesis, suggesting that the multivalency-binding approach is not valid for integrating the potential of aminoglyoside anitibiotics.

The assembly of multivalency binding models has become a general strategy to enhance the binding activity of carbohydrates to the receptor proteins.[1] Many types of carbohydrate cluster models have been designed, in which neoglycoconjugates,[2] glycodendrons,[3,4] and starfish glycoconjugates[5] are widely investigated. The binding activity is highly enhanced by multivalency binding or carbohydrate cluster effect, especially when the receptor protein possesses plural carbohydrate binding sites in a multiple subunit structure. For example, we previously showed that acrylamide copolymers carrying a synthetic globosyltriaoside (Gb3) cluster highly enhanced the activity to trap and neutralize a Shiga toxin (Stx-I) comprised of five B-subunits, each of which has two Gb3 binding sites.[6] Analogous effects have been communicated by many other groups,[4,5] supporting that the assembly of a carbohydrate cluster can significantly integrate the biological potential of synthetic carbohydrates and their mimics.

The strategy based on multivalent binding effect can be extended to biologically active compounds other than carbohydrates as long as the activity arises from species-specific binding to the receptor molecules. For example, Whiteside et al.[7] have just recently communicated highly enhanced activity of a peptide cluster along an acrylamide backbone for neutralizing *Anthrax* toxins. Intense interest is directed to the assembly of multivalent antibiotic compounds since Arimoto et al. reported the first synthesis of a vancomycin-embedded polymer and its highly integrated antibiotic activity.[8] Multivalent antibiotics are expected to provide a promising way to circumvent the problem of antibiotics-resistant microbes like MRSA (methicilin-resistant *Staphylococcus aureus*) and VRE (vancomycin-resistant *enterococci*). This is mainly because some of the multivalent aminoglycoside antibiotics are reported to show tolerance to modifying enzymes like acetyl and phosphotransferases expressed in resistant strains.[9] In the present study, our interest is directed to the development of a convenient chemical way to convert kanamycin A **1** to multivalent models. In the present communication, we describe a general pathway starting from amikacin **2** towards polyvalent kanamycins **5a** and **5b**. The biological property of the polyvalent kanamycins is also communicated.

Kanamycin is one of the most popularly used aminoglycoside antibiotics. The antibiotic activity is thought to originate in its binding to ribosomal rRNA and the inhibition of protein synthesis in a way similar to the case of neomycin and other aminoglycoside antibiotics.[10] Though the multivalent assembly has been extensively studied for neomycins and their homologues,[11] kanamycin has not been targeted yet in related studies. In the synthesis of multivalent kanamycin

*Corresponding author. Tel.: +81-52-789-2488; fax: +81-52-789-2528; e-mail: kobayash@mol.nagoya-u.ac.jp

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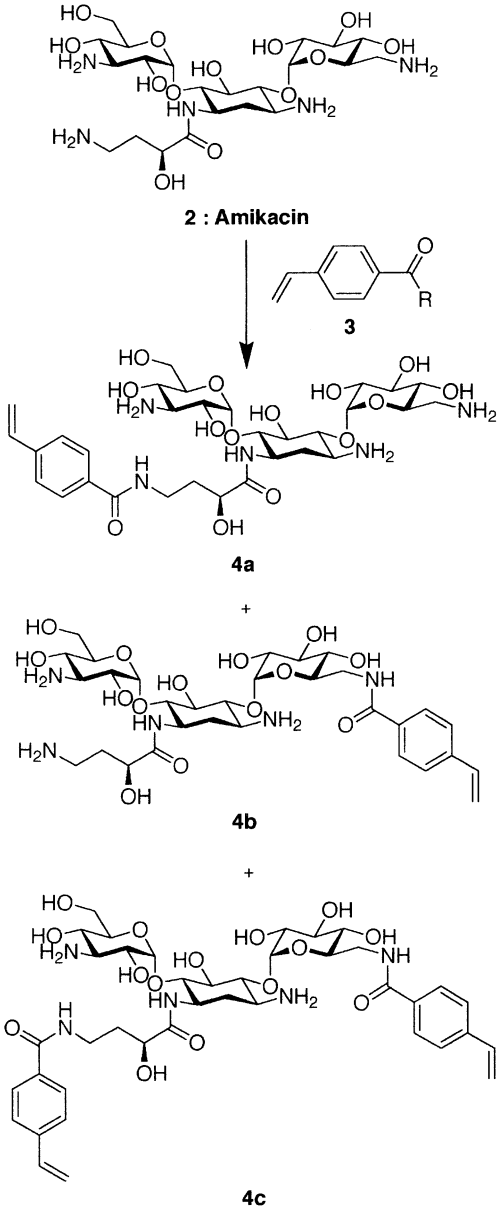


Scheme 1. *N*-*p*-Vinylbenzoylation of amikacin **2**.

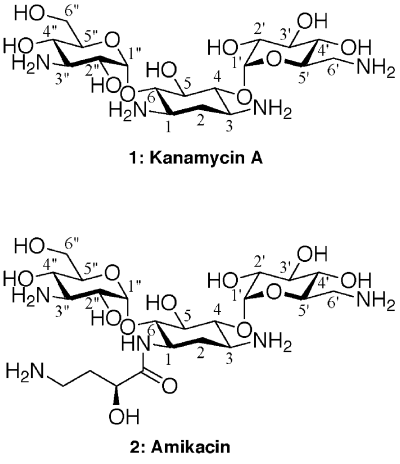
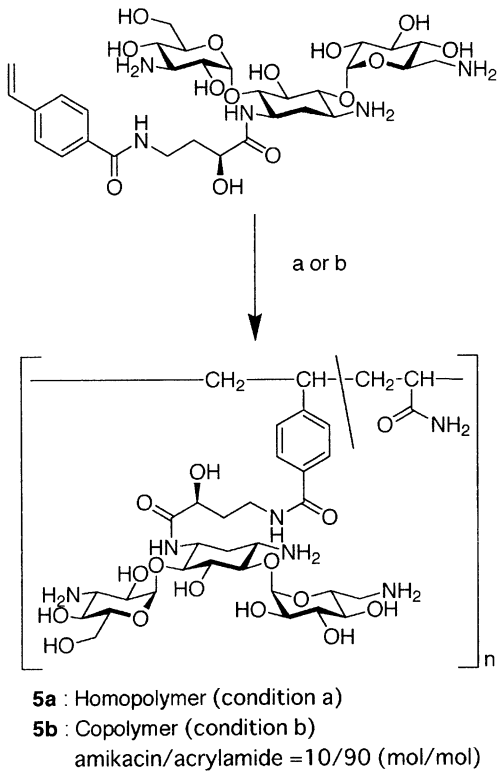


Figure 1. Structures of kanamycin A **1** and amikacin **2**.



Scheme 2. Homo- and co-polymerization of *p*-vinylbenzoylamikacin with acrylamide. Reagents and conditions: (a) 2,2′-azobis (2-amidinopropane) dihydrochloride (1 mol%), H_2O, 60 °C, 8 h, 52%; (b) acrylamide (9 equiv), 2,2′-azobis (2-amidinopropane) dihydrochloride (3 mol%), H_2O, 60 °C, 4 h, 70%.

models, difficulty seems to arise from the differentiation of four amino groups for chemical manipulations. In this study, amikacin **2** (Scheme 1), showing an antibiotic spectrum analogous to that of kanamycin, was employed as a convenient starting compound (Fig. 1). Though amikacin also has four amino groups, an amino group at the side chain [4-amino-2-hydroxy-butanoyl group: AHB group] is known to have the highest basicity[12] suggesting the availability of selective modification at this position. It can also be predicted that the other three amino groups in the core structure are responsible for antibiotic activity as in the case of neamines.[11]

To examine the reactivity of the four amino groups, amikacin **2** was *N*-acylated with a series of *p*-vinylbenzoyl agents **3** carrying different leaving groups [i.e., Cl, *p*-nitrophenoxy, and *O*-(*N*-succinimidyl)]. The identification of the products by ^{1}H NMR and mass spectroscopy allowed us to confirm that the AHB amino group has the highest reactivity to afford **4a** with *p*-vinylbenzamide at the AHB position (Table 1). Another amino group at the primary C-6′ position was also *N*-acylated to give **4b** together with a concomitant amount of di-*N*-acylated amikacin **4c**. The relative ratio of the main products **4a** and **4b** changed slightly depending on the leaving groups. When *p*-vinylbenzoyl chloride was employed, a mixture of **4a** and **4b** was derived in 68:32 ratio (^{1}H NMR and FAB-MS analysis[13]). The use of *p*-nitrophenoxyl, and *O*-(*N*-succinimidyl) groups could improve the selectivity at the AHB

Table 1. *N*-*p*-Vinylbenzoylation of amikacin

Run no.	3		K_2CO_3 equiv	Solvent	Temp. (°C)	Time (h)	Yield (**4a**/**4b**)[a], (%)
	R	Equiv					
1	Chloride	2.0	2.0	THF/H_2O	0–rt	0.5	28 (68/32)
2	*p*-Nitrophenoxy	1.2	2.0	THF/H_2O	rt	2	38 (86/14)
3	*p*-Nitrophenoxy	1.2	2.0	$MeCN/H_2O$	rt	2	42 (85/15)
4	*p*-Nitrophenoxy	1.2	—	$MeCN/H_2O$	rt	2	40 (86/14)
5	*O*-(*N*-Succinimidyl)	1.2	2.0	THF/H_2O	rt	1	54 (90/10)
6	*O*-(*N*-Succinimidyl)	1.2	2.0	$MeCN/H_2O$	rt	1	51 (92/8)
7	*O*-(*N*-Succinimidyl)	1.2	—	$MeCN/H_2O$	rt	1	50 (82/18)

[a]Determined by ^1H NMR.

amino group, in which the *N*-succinimidyl group showed better efficiency for selectivity and yield. Unreacted amikacin **2** could be recovered from the reaction mixture (ca. 25%) and reused after purification on a short ODS column.

A *p*-vinylbenzoyl derivative **4** thus derived was subjected to radical homopolymerization and copolymerization with acrylamide to afford desired polyvalent kanamycin models **5a** ($M_r = 150$ KDa, determined by SEC) and **5b** (kanamycin/acrylamide = 10/90 as determined by ^1H NMR spectroscopy, $M_r = 90$ KDa), respectively (Scheme 2). The acrylamide copolymer **5b** was prepared to examine possible effects of the flexibility of the backbone and the clustering of amikacin at the side chain. Thus, polyvalent kanamicin models could be derived starting from amikacin in a facile way without the tedious protection and deprotection processes.

Antibiotics activity was assayed for the monomeric ligand **4** and the polyvalent models **5a** and **5b**. An authentic micro-dilution assay revealed that the monomeric ligand **4** shows activity at 40–60 μg/mL against *S. aureus*, *Escherichia coli,* and *P. aeruginosa,* indicating that the modification at the AHB group did not fatally affect the antibacterial activity. On the other hand, none of the polyvalent models **5a** and **5b** showed apparent activity at 100 μg/mL against these microbes as well as against other species, *Staphilococcus epidermidis, Enterococcus hirae, Enterococcus faecalis, Klebsiella pneumoniae, Citrobacter freundii, Enterobacter cloacae,* and *Serratia marcescens*. It is apparent, therefore, that the assembly of polyvalent kanamycins results in the complete loss of antibacterial activity. The result is contraty to our expectation and the concept of multivalency binding effects, suggesting that activity enhancement is not always achieved for multivalent binding models.

In the present case, the polymeric compounds **5a** and **5b** seemed to hardly internalize into bacterial cell walls even though they might possess enhanced activity for rRNA binding to inhibit protein synthesis. In order to verify this expectation, an in vitro assay using *E. coli* S30-extract and green-fluorescence plasmid DNA system[15] was performed for **4**, **5a**, and **5b**. Under the conditions that the monovalent ligand **4** inhibited the protein synthesis ($IC_{50} = 5$ μM), however, none of the polyvalent models **5a** and **5b** showed inhibitory activity ($IC_{50} > 100$ μM). This has revealed that the assembly of polyvalent models results in the complete loss of antibacterial activity even in the inhibition of protein synthesis.

The results in Table 1 apparently conflict with the current topic of applying multivalent models for antibiotics. The loss of antibacterial activity may be due to the steric factor of high molecular-weight polymers **5a** and **5b**. The conformation of the polymer backbone and/or the shortage in the linker length may not allow kanamycin at the side chain to access to the A-site of the rRNA chain responsible for protein synthesis. It is also possible that the polyvalent aminoglycosides may have altered binding sites to those other than the rRNA A-site. In this case, the strategy based on polyvalent binding effects will be hardly applicable to aminoglycoside antibiotics, and the approach based on di- and trivalent binding models may be more effective. Such comparison is under investigation with the synthesis of di- and tri-valent kanamycins from the *N*-benzoylated amikacin derivatives and the results will be reported in due course.

In conclusion, we have presented the first synthesis of polyvalent kanamycin models and examined their antibacterial activity. It has been also found that the polyvalent models have lost the antibacterial activity of amikacin. The result strongly suggests that the activity enhancement by polyvalency binding models may not be extended to aminoglycoside antibiotics. The result, however, does not rule out the potential utility of polymer-based aminoglycosides. For example, they have biological potential as polymer prodrugs[16] and also as polycationic vectors for drug and DNA delivery.[17] Such applications will be investigated in our group along with the design of other multivalent aminoglycoside antibiotics.

References and Notes

1. Bovin, N. V.; Gabius, H.-J. *Chem. Soc. Rev*. **1995**, *24*, 413.
2. Fulton, D. A.; Stoddart, J. F. *Bioconjugate Chem*. **2001**, *12*, 655.
3. Zanini, D.; Roy, R. *J. Am. Chem. Soc*. **1997**, *119*, 2088.
4. Matsuoka, K.; Terabatake, M.; Esumi, Y.; Terunuma, D.; Kuzuhara, H. *Tetrahedron Lett*. **1999**, *40*, 7839.
5. Kitov, P. I.; Sadowska, J. M.; Mulvey, G.; Armstrong,

G. D.; Ling, H.; Pannu, N. S.; Read, R. J.; Bundle, D. R. *Nature* **2000**, *403*, 669.
6. Dohi, H.; Nishida, Y.; Mizuno, M.; Shinkai, M.; Kobayashi, T.; Takeda, T.; Uzawa, H.; Kobayashi, K. *Bioorg. Med. Chem.* **1999**, *7*, 2053.
7. Ravis, M. M.; Mogridge, S. J.; Metallo, S.; Deschatelets, P.; Sellman, B. R.; Whiteside, G. M.; Collier, R. J. *Nat. Biotechnol.* **2001**, *19*, 958.
8. (a) Arimoto, H.; Nishimura, K.; Kimuni, T.; Hayakawa, I.; Uemura, D. *Chem. Commun.* **1999**, 1361. (b) Arimoto, H.; Oishi, T.; Nishijima, M.; Kimuni, T. *Tetrahedron Lett.* **2001**, *42*, 3347.
9. Sucheck, S. J.; Wong, A. L.; Koeller, K. M.; Boehr, D. D.; Draker, K.; Sears, P.; Wright, G. D.; Wong, C.-H. *J. Am. Chem. Soc.* **2000**, *122*, 5230.
10. Formy, D.; Recht, M. I.; Blanchard, S. C.; Puglisi, J. D. *Science* **1996**, *274*, 1367.
11. (a) Wang, H.; Tor, Y. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1951. (b) Tok, J. B.-H.; Dunn, L. J.; Jean, R. C. D. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1127. (c) Tok, J. B.-H.; Fenker, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2987.
12. Kane, R. S.; Glink, P. T.; Chapman, R. G.; McDonald, J. C.; Jensen, P. K.; Gao, H.; Pasa-Tolicacute, L.; Smith, R. D.; Whiteside, G. M. *Anal. Chem.* **2001**, *73*, 4028.
13. Though separation of **4a** and **4b** from the di-benzoylated amikacin **4c** could be performed easily by a reversed-phased C-18 column chromatography, the two regioisomers **4a** and **4b** could be hardly separated even on HPLC columns. Though ^{1}H NMR data of the mixture of **4a** and **4b** did not enable us to determine the structure of the minor component, the ^{1}H NMR assignment of C6′-*N*-*p*-nitrobenzoyl kanamycin allowed us to assign its structure as C6′-*N*-*p*-nitrobenzoyl derivative **4b**. The ^{1}H NMR signals of the main product **4a**, compared with those of amikacin,[14] showed that the *N*-benzoylation caused chemical shift changes for the C–H protons in the side group without causing apparent change in the core protons.
14. Gaggeli, E.; Gaggelli, N.; Maccotta, A.; Valensin, G.; Marini, D.; Cocco, M. E. D.; Delfini, M. *Spectrochim. Acta Part A* **1995**, *51*, 1959.
15. Inhibition activity was determined by a decrease of fluorescence intensity of variant green fluorescence protein (sgGFP: Ex = 474 nm, Em = 509 nm) that was transcribed and translated from plasmid DNA (pQBI T7-GFP) by in vitro transcription and translation kit (Ambion PROTEINscript-PRO™). IC_{50} of amikacin was about 0.1 μM in this method.
16. Zovko, M.; Zork, B.; Lovrek, M.; Boneschans, S. *Int. J. Pharm.* **2001**, *228*, 129.
17. Godbey, W. T.; Wu, K. K.; Mikos, A. G. *J. Control. Release* **1999**, *60*, 149.