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Solid-phase synthesis of peptide ribonucleic acids (PRNA)

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Abstract—The range of available peptide ribonucleic acid (PRNA) monomers was fully expanded for the use in solid-phase synthesis of PRNA oligomers, which were designed to reversibly control the recognition and complexation behavior of the complementary DNA/RNA by external factors. A couple of PRNA 12-mers with desired purine–pyrimidine mixed sequences were prepared indeed in high yields by the solid-phase synthesis.
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1. Introduction

The completion of the human genome sequencing project[1] has driven our research interest and endeavor to the creation of gene therapeutic drugs using the antisense and antigene strategies. Thus, a number of nucleic acid derivatives and analogues have been proposed for the use in gene therapy, for which both the resistance to nuclease, and the enhanced hybridization affinity[2] are required for the oligomers as well as the resulting hybrids. Peptide nucleic acid (PNA), originally proposed by Nielsen,[3] is one of the most successful nucleic acid analogues, which employ an oligo[*N*-(2-aminoethyl)glycine] backbone instead of the ribose–phosphate backbone in natural nucleic acid. However, these hitherto reported nucleic acid derivatives and analogues, including PNA, have an inherent limitation or drawback, lacking an ability to actively control the function of these nucleic acids by external stimuli.

We have recently proposed a new category of peptide ribonucleic acid (PRNA), in which the 5′-amino-5′-deoxypyrimidine ribonucleoside moiety is appended to an oligo(γ-L-glutamic acid) backbone through the 5′-amino group[4] (Chart 1).

Possessing improved solubility in water, longer ribose tether, and matched helical pitch, γ-PRNA 8-mers with an isopoly(L-glutamic acid) backbone form a stable complex with complementary DNAs. Furthermore, the recognition and complexation behavior of γ-PRNA 8-mers with target DNAs can be controlled by borax added as an external factor through the 2′,3′-borate formation.[4] Such an active

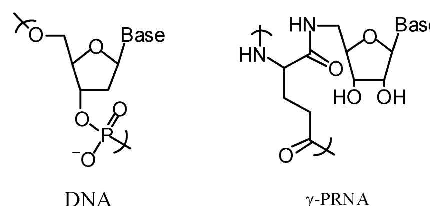

Chart 1.

control of DNA recognition by external factor has not been achieved and hence the PRNA approach is certainly useful as a unique method to realize the 'on-demand' gene therapeutic drug in the antisense strategy and may find various biochemical and pharmaceutical applications as a powerful and versatile tool. However, the range of available PRNA is quite limited in variety at present, only simple 8-mers of homo-uracil sequence and of alternating uracil–cytosine sequence having been synthesized by fragment condensation method in solution.[4] To further expand the scope and also to demonstrate the general validity of the PRNA strategy, it is absolutely necessary to establish simpler-and-versatile synthetic routes not only to the PRNA monomers of all four purine and pyrimidine nucleobases but also to 10 to 20-meric PRNA oligomers of purine–pyrimidine mixed sequences.[2]

In an effort to develop a simpler and more effective route to oligo-PRNAs, we found that the solid-phase peptide synthesis technique is applicable to the PRNA oligomer synthesis. In this strategy, 9-fluorenemethyloxycarbonyl (Fmoc), which is readily removable under mild basic conditions,[5] was employed as the protective group for the N-terminus of PRNA monomer, while benzoyl (Bz) group for the exocyclic amino residues of nucleobases.[6] In this paper, we report the syntheses of four Fmoc-protected peptide ribonucleic acid monomers with uracil,

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N-Bz-cytosine, hypoxanthine, and *N*-Bz-adenosine nucleobases, which were subsequently used in the solid-phase syntheses of a couple of PRNA 12-mers with desired purine–pyrimidine mixed sequences.

2. Results and discussion

Most of the existing antisense molecules are designed to simply inhibit the genetic information transfer, while little effort has been devoted to the active control of the DNA/RNA recognition processes.[2] In our previous studies,[4,7] we have proposed a new methodology and effective tools for controlling DNA/RNA recognition by external agent. This strategy employs a novel nucleic acid analogue, i.e. γ-peptide ribonucleic acid (γ-PRNA), as the recognition moiety with a built-in switch triggered by an external agent. The borate ester formation of ribose's *cis*-2′,3′-diol and the synchronized hydrogen-bonding interaction between the ribose's 5′-amide proton and the 2-carbonyl oxygen of nucleobase act as the external and internal switching devices (Scheme 1). Furthermore, we have demonstrated for the first time that stable complexes of γ-PRNA with complementary oligonucleotides are readily dissociated by adding borax. Thus, PRNA is certainly one of the most promising antisense molecules of the next generation and can be used as a powerful and versatile tool for switching biological functions.

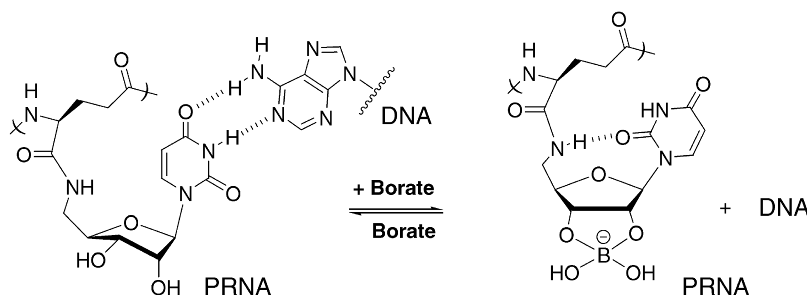
In this study, we applied the solid-phase peptide synthesis technique to the construction of the peptide backbone of PRNA oligomer. In this strategy, we employed the Fmoc/benzotriazol-1-yloxytris (dimethylamino) phosphoniumhexafluoro-phosphate) (Bop), rather than *tert*-butyloxycarbonyl (Boc)/Bop, pair for the protection of N-termini, since the glycosyl bond of nucleoside derivatives is acid-sensitive[8] and the unprotected *cis*-2′,3′-hydroxyl groups of PRNA is less stable under acidic condition.[9] The Fmoc protection allows us to use the newly developed resin with a long ethylene glycol spacer (NovaSyn® TRG resin), which is known to give high coupling yields particularly in the syntheses of bulky long peptides,[10] whereas the Boc protection requires the use of conventional, but somewhat unstable, oxime resin as solid support, which is however somewhat unstable and less reliable in some cases.[11] We first prepared the full set of Fmoc-protected PRNA monomers, which are the essential building blocks for the PRNA synthesis of desired sequence.

2.1. Preparation of 5′-amino-5′-deoxynucleosides

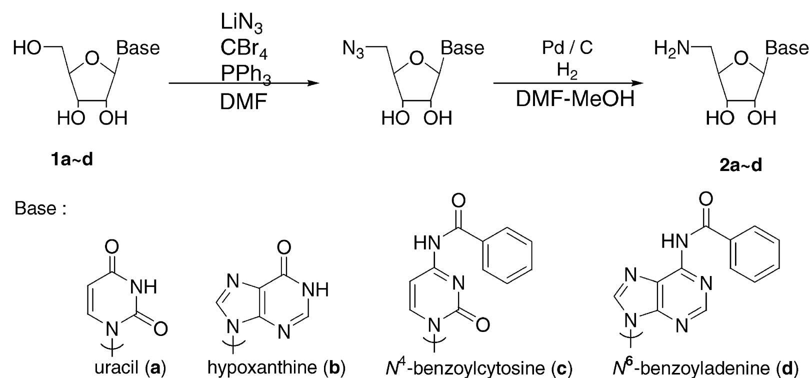
In this study, adenosine, cytidine, uridine, and inosine were used as nucleic acid-recognizing moieties of PRNA. Inosine, lacking the 2-amino group, is a substitute for the guanosine nucleoside of natural nucleic acids and is also a complementary nucleobase of cytosine. 5′-Azido-5′-deoxynucleosides were prepared by reacting the corresponding nucleosides with carbon tetrabromide and triphenylphosphine in the presence of lithium azide.[12] The exocyclic amino group of adenosine and cytidine was protected by a benzoyl group,[6] which can survive the catalytic hydrogenation to deprotect the benzyl group and also the trifluoroacetic acid (TFA) treatment to deprotect the Boc group, yet be removed by the treatment with 28% aqueous ammonia. The nucleosides with and without protected amino group were converted to 5′-azido-5′-deoxy-nucleosides in moderate to high yields (56–90%). 5′-Azido-5′-deoxyuridine (5′-N_3-Urd) and -inosine (5′-N_3-Ino) were purified by column chromatography on silica gel, while exocyclic amino-protected 5′-azido-5′-deoxycytidine (5′-N_3-Cyd(N^4-Bz)) and -adenosine (5′-N_3-Ado(N^6-Bz)) by recrystallization from ethanol. The azido residue of 5′-azido-5′-deoxynucleoside was converted to an amino group by catalytic hydrogenation.[12] These 5′-azido-5′-deoxynucleosides were readily hydrogenated in quantitative yields under a mild condition. The 5′-amino-5′-deoxynucleosides thus obtained were purified by reprecipitation from methanol by adding ether (Scheme 2).

2.2. Preparation of *N*-Boc-protected γ-PRNA monomers

PRNAs, in which 5′-amino-5′-deoxyribonucleoside moiety is appended to each amino acid residue of the oligo(γ-L-glutamic acid) backbone, are designed not only to enhance the hybridization affinity as well as the stability of antisense molecule and its hybrid in cytosol, but also to reversibly control the recognition behavior by external factors. In PRNA monomers, 5′-amino-5′-deoxyribonucleoside unit is tethered to the α-carboxyl of α-amino/γ-carboxyl-protected L-glutamic acid through the 5′-amino group, thus reserving the ribose's *cis*-2′,3′-diol for the cyclic borate formation and the 5′-amide proton for the hydrogen-bonding interaction with the pyrimidine's 2-carbonyl. Since the treatment with diisopropylethylamine (DIEA) is unavoidable in the synthesis of PRNA monomer, Boc-protected L-glutamic acid was chosen as the starting compound (as the Fmoc protective group is unstable even under mild basic conditionsa). In all PRNA monomer



Scheme 1. Proposed recognition control model of PRNA with complementary DNA.



Scheme 2.

syntheses, *N*-Boc-L-glutamic acid γ-benzyl ester (Boc-Glu(OBzl)-OH) was condensed with 5′-amino-5′-deoxy-nucleosides in the presence of Bop reagent and 1-hydroxy-benzotriazole (HOBt) as condensation reagents. The protected PRNA monomers showed solubilities in methanol varying from highly soluble Boc-isoGln(5′U)-OBzl (**3a**) and Boc-isoGln(5′A(N^6-Bz))-OBzl (**3d**) to less soluble Boc-isoGln(5′I)-OBzl (**3b**), and then to slightly soluble Boc-isoGln(5′C(N^4-Bz))-OBzl (**3c**). In particular, **3c** could not be subjected to open column chromatography. Hence, the PRNA monomers containing uracil (**3a**), hypoxanthine (**3b**), and adenine (**3d**) were purified by column chromatography on silica gel, while the cytosine-containing PRNA monomer (**3c**) was purified by reprecipitation from ether with methanol (Scheme 3).

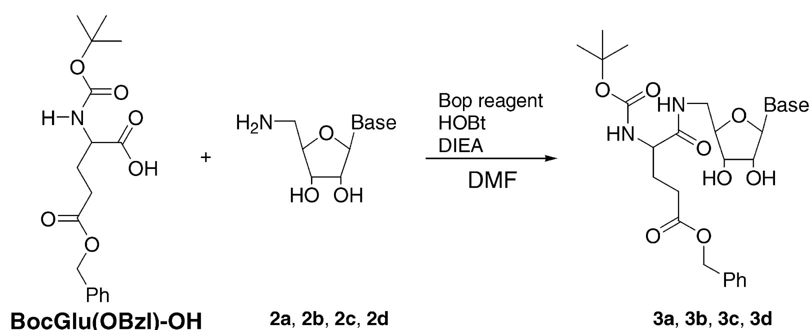
2.3. Preparation of *N*-Fmoc-protected γ-PRNA monomers

Fmoc, which is readily removable under mild basic conditions, is one of the most frequently employed α-amino-protecting groups in solid-phase peptide synthesis. An alternative conventional solid-phase peptide synthesis employs Boc as the protective group for the N-terminus and trifluoroacetic acid (TFA) as the deprotecting reagent. However, this method often causes depurination of purinenucleoside derivatives upon treatment with TFA.[7] We therefore used the Fmoc method in the solid-phase synthesis of PRNAs.

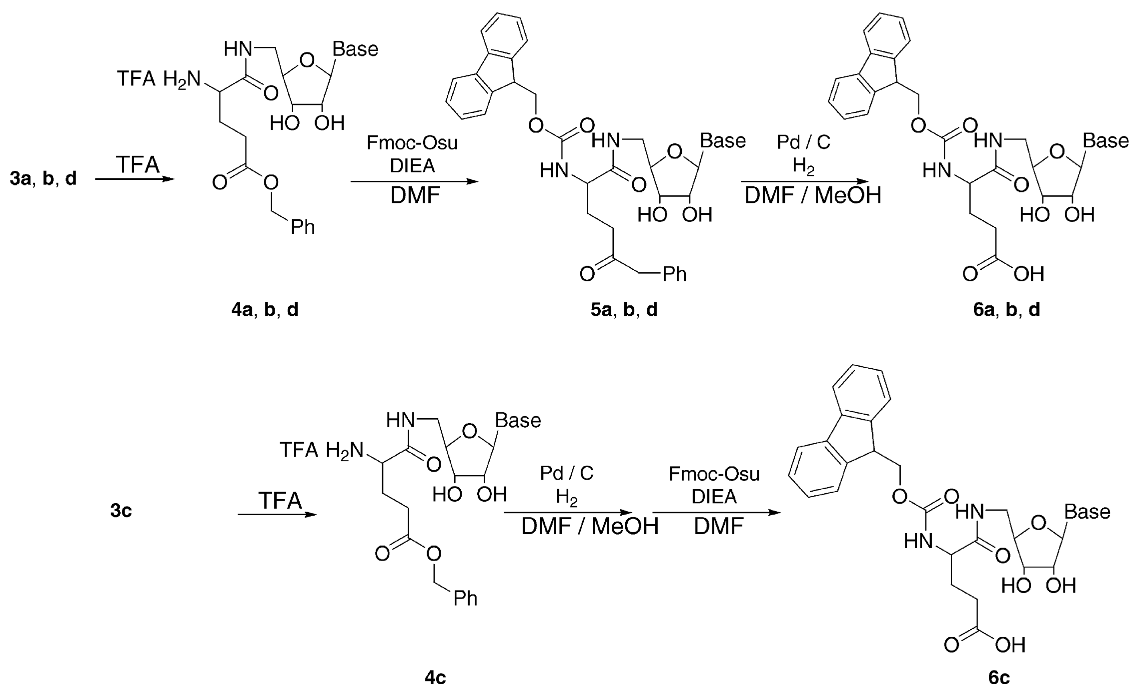
Fmoc-protected PRNA monomers (**6a–d**) were prepared from the corresponding Boc-PRNA-OBzl monomers **3a–d**. The Boc group of **3a–d** was removed by the treatment with TFA at room temperature to give the TFA salts of PRNA-OBzl monomers (**4a–d**). Then, these PRNA-OBzl monomers, possessing a free amino group at the α-position, were reacted with *N*-(9-fluorenylmethyloxycarbonyloxy) succinimide (Fmoc-OSu) to give Fmoc-protected PRNA-OBzl monomers (**5a–d**). Fmoc-isoGln(5′U)-OBzl **5a** was purified by reprecipitation from a methanol solution by adding diethyl ether, while Fmoc-isoGln(5′I)-OBzl (**5b**) and Fmoc-isoGln(5′A(N^6-Bz))-OBzl (**5d**) were purified by short column chromatography. The Bzl protection of Fmoc-PRNA-OBzl monomers was quantitatively removed by catalytic hydrogenation over palladium/charcoal to afford the corresponding Fmoc-PRNA monomers, carrying a free carboxylic acid at the γ-position for solid-phase peptide synthesis. Since the Fmoc-isoGln(5′C(N^4-Bz))-OBzl (**4c**) showed poor solubility in DMF (although the fully deprotected 5′C(N^4-Bz)-PRNA monomer was sufficiently soluble in DMF), then we employed a different approach to Fmoc-isoGln(5′C(N^4-Bz))-OH (**6c**). In this strategy, the Bzl group of **4c** was first removed by catalytic hydrogenation, and then the Fmoc group was introduced by reaction with Fmoc-OSu to give **6c**. Now, a full set of the four Fmoc-protected PRNA monomers with a free carboxyl group (Fmoc-isoGln(5′N)-OH (**6a–d**) were prepared in moderate overall yields (Scheme 4). The obtained Fmoc-PRNA monomers were soluble and stable enough to be handled in *N*-methylpyrrolidone (NMP),[13] a representative solvent employed in solid-phase peptide synthesis.

2.4. Solid-phase synthesis of PRNA oligomers

First of all, the compatibility of Fmoc/Bz-protected PRNA monomers with the standard solid-phase peptide synthesis protocol (Chart 2) was checked. It was thus examined whether or not the Bz-protected exocyclic amino residue of



Scheme 3.



Scheme 4. Preparation of Fmoc-protected PRNA monomers (**6a–d**).

nucleobase in PRNA monomers can survive the piperidine treatment used in the Fmoc deprotection. HPLC and UV spectral analyses indicated that both PRNA monomers, containing N^4-benzoylcytosine and N^6-benzoyladenine, are resistant to the piperidine treatment at ambient temperature, and that the Bz protection is efficiently removed in quantitative yield upon treatment with 28% aqueous ammonia. It was further demonstrated that the conventional solid-phase synthesis protocol does not damage the ribose's unprotected *cis*-2′,3′-diol of PRNA monomers appended to the polymer support. Thus, practically no side reactions, such as esterification, were found to occur in the oligomeric PRNAs recovered from the solid support. These results clearly indicate that oligomeric PRNAs can be prepared from the Fmoc/Bz-protected PRNA monomers using the standard solid-phase peptide synthesis protocol.

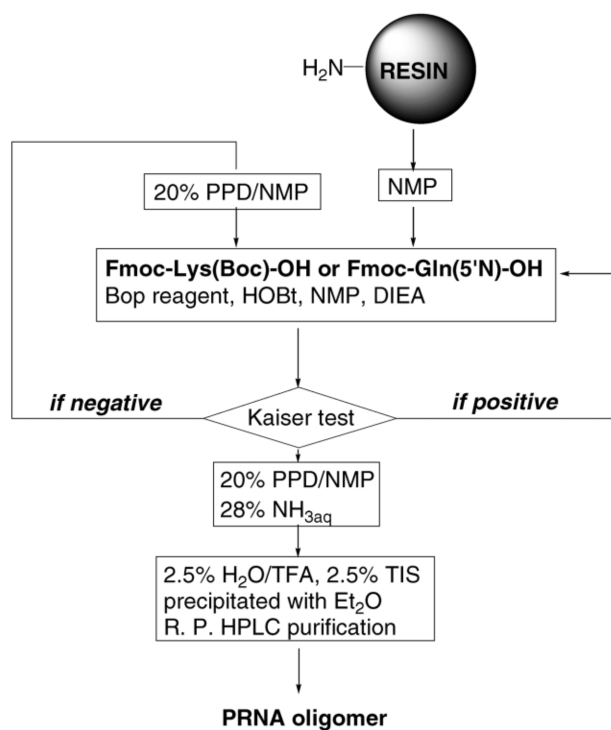


Chart 2. Solid-phase peptide synthesis using Fmoc protective group.

In this study to prepare a series of long-tethered oligomeric PRNAs, we employed a newly developed solid support with a long oxyethylene spacer (NovaSyn® TRG resin), which was shown to give high coupling yields particularly in the synthesis of bulky or long peptides.[7] Fmoc-PRNA monomer (3 equiv.), Bop reagent (3 equiv.), HOBt (3 equiv.), and DIEA (6 equiv.) were added to an NMP slurry of resin (1 equiv.), and the peptide backbone was elongated step by step (Chart 2). The Fmoc protective group was removed by the treatment with 20–30% piperidine in NMP. In each coupling step, the backbone elongation was confirmed by the conventional Kaiser test and the UV detection of Fmoc–piperidine adduct. If the result of Kaiser test was positive, the coupling reaction was repeated 2–3 times until the Kaiser test became negative and the Fmoc–piperidine adduct was formed quantitatively. All of the four PRNA monomers displayed excellent coupling efficiency and the quantitative coupling was achieved even in the synthesis of 12-mers, as demonstrated below. In the final step of the solid-phase synthesis, the Bz protection at the exocyclic amino group of adenosine and cytosine was removed by the treatment with 28% aqueous ammonia. After being thoroughly washed with NMP and chloroform, the target PRNA oligomer synthesized on the solid support was cleaved and isolated from the resin by TFA containing 2.5% water and 2.5% triisopropylsilane as scavengers. The crude PRNA oligomers in TFA were reprecipitated by adding cold ether to give TFA salts of PRNA oligomers as white powder. The PRNA oligomers thus obtained were purified by reversed phase preparative HPLC. Excellent yields of up

to 98.5%/step were obtained and no byproduct was detected, while lower coupling yields around 80% were reported for the solution-state fragment condensation synthesis.

A couple of PRNA 12-mers, which contain either uracil and hypoxanthine or uracil, adenine, and cytosine, were synthesized indeed. Thus, PRNA 12-mer NH_2-CCU-UAC-UAU-CUC-Lys-*OH* (**7**) was prepared by the Fmoc solid-phase synthesis on the newly developed solid support with an amino residue content of 0.21 mmol/g. In the first step, the solid support (100 mg) was suspended in an NMP solution of Fmoc-Lys(Boc)-OH (29.5 mg, 0.63 mmol), Bop reagent (28.0 mg, 0.63 mmol, 1 equiv.), and HOBt (12.2 mg, 0.63 mmol, 1 equiv.), to which was added DIEA (23.1 μL, 1.26 mmol, 2 equiv.), and then the resulting solution was gently stirred at room temperature. After 15 min of stirring, the resin was washed 5 times with NMP, and if the Kaiser test was negative Fmoc was removed by treating the Lys(Boc)-appended resin with 30% piperidine in NMP for 15 min. After the deprotection, the resin was washed 5 times with NMP. The same procedure was repeated until the PRNA 12-mer of desired sequence was obtained by using the appropriate Fmoc-PRNA monomers, i.e. Fmoc-isoGln(5′U)-OH (37.5 mg, 0.63 mmol), Fmoc-isoGln(5′A(N^6-Bz)-OH (45.5 mg, 0.63 mmol), and Fmoc-isoGln(5′C(N^4-Bz)-OH (44.0 mg, 0.63 mmol). After the 12-mer was reached, the resin was washed with water and the benzoyl protection was removed by the treatment with 28% aqueous ammonia, and finally the resin was thoroughly washed with NMP and chloroform. After drying under a reduced pressure, the total resin weight was measured and the crude yield of PRNA 12-mer was determined as 83.4% (98.5%/step). A consistent yield of 82% was obtained from the UV spectrometric determination of ammonium benzoate solution obtained in the deprotection process of the solid-phase PRNA synthesis. The deprotected PRNA 12-mer was cleaved from the resin by TFA treatment and purified by reversed phase preparative HPLC eluted with aqueous acetonitrile. HPLC fractions, containing the PRNA 12-mer, were collected and the combined fraction was freeze-dried to give the target PRNA 12-mer, NH_2-CCU-UAC-UAU-CUC-Lys-*OH* (**7**), as white powder. The isolated PRNA 12-mer was analyzed by analytical HPLC on an RP18 column to show a single peak (>99% pure); see Figure 1. MALDI-TOF mass spectrometric analysis (negative mode) also indicated a >98% purity of PRNA 12-mer (found *m*/*z* 4436.51 (M); Calcd 4436.65).

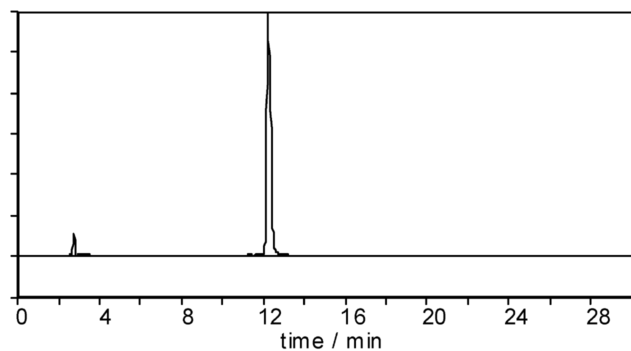


Figure 1. HPLC trace of PRNA 12-mer (**8**), purified on a preparative ODS column (acetonitrile/H_2O=9/91).

Similarly, the self-complementary PRNA 12-mer, NH_2-III-CCI-CII-CCC-Lys-*OH* (**8**), was obtained in good yield (88.0%, 98.4%/step). The purity was checked with ^{1}H NMR and MALDI-TOF MS (positive mode; Found *m*/*z* 4535.15 (M+1); calcd 4533.68).

3. Conclusion

We established the synthetic routes to a series of PRNA monomers, carrying adenine, cytosine, hypoxanthine, and uracil nucleobases as recognition sites. This enabled us to fully expand the range of available PRNA monomers. We further demonstrated that the newly synthesized Fmoc-protected PRNA monomers are compatible with the standard solid-phase peptide synthesis protocol, and oligomeric PRNAs with purine–pyrimidine mixed sequences can be prepared. Indeed, representative PRNA 12-mers of mixed sequences were synthesized in high yields by the Fmoc solid-phase peptide synthesis, and were characterized by HPLC and MALDI-TOF mass spectrometric analyses. It is noted that the solid-phase synthesis is much more convenient, efficient, and reliable than the conventional fragment condensation method in solution reported previously. The present synthetic strategy should lead us to a wide variety of PRNA oligomers with desired sequences, which function as reversibly controllable antisense molecules upon complexation with the complementary DNAs. Studies along this line are currently in progress.

4. Experimental

4.1. General

Standard abbreviations for amino acids and protecting groups are as recommended by the IUPAC-IUB Commission on Biochemical Nomenclature.

All starting materials, reagents, and solvents were commercially available and used without further purification. Nucleosides were purchased from Seikagaku Co. Ltd. (Tokyo, Japan). Boc-L-amino acids, Fmoc-L-amino acids, and 1-hydroxybenzotriazole (HOBt) were purchased from Peptide Institute, Inc. (Osaka, Japan). Other chemicals of guaranteed grade were purchased Tokyo Kasei Kogyo Co. (Tokyo, Japan) or Wako Pure Chemical Industries Co. Ltd. (Osaka, Japan). Column chromatography was performed on silica gel (70–230 mesh) from Kanto Kagaku. Gel filtration was performed on Sephadex G-25 from Pharmacia. Nucleic acid analogues were purified on an ODS column by elution with 10% acetonitrile in water at a flow rate of 3 mL/min.

IR spectra were recorded on a JASCO FT/IR-230 spectrometer. UV spectra were recorded on a JASCO V-550 UV/vis spectrophotometer equipped with a temperature controller. ^{1}H NMR spectra were obtained on a JEOL GSX-270 at 270 MHz or a Varian INOVA-600 at 600 MHz. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (δ_H=0.0 ppm) as internal standard. Mass spectral measurements were performed on a JEOL AX-500 instrument by fast-atom bombardment (FAB) ionization with nitrobenzyl alcohol (NBA) as a

matrix or on a Voyager RP from PerSeptive Biosystems with α-cyano-4-hydroxycinnamic acid (α-CHCA) or picolinic acid as a matrix (MALDI-TOF).

4.1.1. N^{α}-Fluorenylmethyloxycarbonyl-N^5-(5′-deoxy-5′-uridyl)-L-isoglutamine (Fmoc-isoGln(5′U)-OBzl) (6a). N^5-(5′-Deoxy-5′-uridyl)-L-isoglutamine benzyl ester trifluoroacetic acid salt (TFA·isoGln(5′U)-OBzl) was synthesized as described previously.[4] To a solution of TFA·isoGln(5′U)-OBzl (2.20 g, 3.81 mmol) and 9-fluorenylmethyl *N*-succinimidyl carbonate (Fmoc-OSu) (1.54 g, 4.57 mmol) in DMF (100 mL), diisopropylethylamine (1.19 g, 7.62 mmol) was added at 0°C. The mixture was stirred for 10 min at room temperature. The solvent was removed under a reduced pressure and methanol was added to the residue to precipitate Fmoc-isoGln(5′U)-OBzl (**5a**) as a white precipitate, which was filtered, washed with methanol, and dried under a reduced pressure to give the product (2.16 g, 82.8%). A catalytic amount of 10% Pd/C (ca. 0.1 g) was added to a solution of Fmoc-isoGln(5′U)-OBzl (2.16 g, 3.15 mmol) in methanol–DMF mixture (1:1 v/v). The mixture was stirred for 2 h under a hydrogen atmosphere, and then the catalyst was filtered. The filtrate was concentrated by rotary evaporation under a reduced pressure, to which a small amount of methanol and then ether were added to give the title compound as white powder (2.08 g, 3.12 mmol).

4.1.2. Fmoc-isoGln(5′U)-OBzl (5a). ν_{max} (KBr disc)/cm^{-1} 3320, 3070, 3950, 1690, 1530, 1450, 1400, 1320, 1280, and 1200; δ_H (270 MHz; DMSO-d_6) 1.69–2.00 (2H, m, β-CH_2), 2.37, (2H, t, γ-CH_2), 3.20–3.44 (2H, m, 5′-H), 3.75–3.89 (2H, m, fluorene–CH_2–O), 3.97–4.08 (2H, m, 3′, 4′), 4.15–4.30 (3H, m, α, 2′, fluorene methine), 5.07 (2H, s, OCH_2–Ph), 5.17–5.30 (1H, bs, 3′-OH), 5.38–5.50 (1H, bs, 2′-OH), 5.61 (1H, d, 5-H), 5.73 (1H, d, 1′-H), 7.25–7.88 (15H, m, fluorene (8H), α-N*H*CO (1H), benzyl (5H), 6-H (1H)), 8.13 (1H, t, 5′-NH), 11.34 (1H, s, 3-NH). Anal. found: C, 63.18; H, 5.39 N, 8.10. Calcd for $C_{36}H_{36}N_4O_{10}$: C, 63.15; H, 5.30; N, 8.18; FAB MS. Found *m/z* 685 (M+1), calcd 684.24.

4.1.3. Fmoc-isoGln(5′U)-OH (6a). ν_{max} (KBr disc)/cm^{-1} 3320, 3060, 2940, 1700, 1540, 1450, 1390, 1260, and 1100; δ_H (270 MHz; DMSO-d_6) 1.67–2.00 (2H, m, β-CH_2), 2.29, (2H, t, γ-CH_2), 3.20–3.48 (2H, m, 5′-H), 3.73–3.89 (2H, m, fluorene–CH_2–O), 3.96–4.09 (2H, m, 3′, 4′), 4.16–4.32 (3H, m, α, 2′, methine of fluorene), 5.23–5.51 (2H, bs, 2′-OH, 3′-OH), 5.63 (1H, d, 5-H), 5.73 (1H, d, 1′-H), 7.25–7.76 (10H, m, fluorene (8H), α-NHCO (1H), 6-H (1H)), 8.10 (1H, t, 5′-NH), 11.35 (1H, s, 3-NH); HR-FAB MS. Found *m/z* 595.2015 (M+1, error [ppm/mmu, −4.2/−2.5]), calcd 595.2040 (M+H).

4.1.4. N^{α}-Fluorenylmethyloxycarbonyl-N^5-(5′-deoxy-5′-cytidyl(N^4-benzoyl))-L-isoglutamine (Fmoc-isoGln(5′C(N^4-Bz)-OH) (6c). TFA·isoGln(5′C(N^4Bz))-OBzl was synthesized as described previously.[4] A solution of NH_2-isoGln(5′C(N^4Bz))-OBzl (3.20 g, 4.71 mmol) in DMF–methanol (1:1 v/v) (500 mL) was vigorously stirred under a hydrogen atmosphere with 10% Pd/C (ca. 0.3 g) for 6 h. The mixture was filtered and evaporated under a reduced pressure. The residue was precipitated from ether to give 2.00 g of TFA·NH_2-isoGln(C(N^4-Bz))-OH as a white amorphous solid. To a solution of TFA·NH_2-isoGln(C(N^4-Bz))-OH (2.00 g) and 9-fluorenylmethyl *N*-succinimidyl carbonate (Fmoc-OSu) (1.36 g, 4.03 mmol) in DMF (100 mL), diisopropylethylamine (526, 4.20 mmol) was added at 0°C. The mixture was continuously stirred for 10 min at room temperature. The solvent was evaporated under a reduced pressure and methanol was added to the residue to precipitate Fmoc-isoGln(C(N^4-Bz))-OH as white powder. The powder was filtered and washed with methanol and dried under a reduced pressure to give the title compound (1.40 g, 61%): ν_{max} (KBr disc)/cm^{-1} 3300, 3060, 2930, 1700, 1660, 1560, 1490, 1390, 1320, 1260, 1150, and 1090; δ_H (270 MHz; DMSO-d_6) 1.67–2.00 (2H, m, β-CH_2), 2.26, (2H, t, γ-CH_2), 3.40–3.55 (2H, m, 5′-H), 3.79–4.13 (4H, m, fluorene–CH_2–O, 3′, 4′), 4.16–4.31 (3H, m, α, 2′, methine of fluorene), 5.19 (1H, d, 3′-OH), 5.52 (1H, d, 2′-OH), 5.79 (1H, d, 1′-H), 7.25–8.22 (17H, m, fluorene (8H), α-NHCO (1H), 6-H (1H), 5-H (1H), *o*, *m*, *p*-H (5H), 5′-NH (1H)), 11.27 (1H, s, 4-NHCO); HR-FAB MS. Found *m/z* 698.2469 (M+1, error [ppm/mmu, +1.0/+0.7]), calcd 698.2462 (M+H).

4.1.5. 5′-Azido-5-deoxy-inosine (5′-N_3-Ino). Inosine (890 mg, 3.0 mmol), triphenylphosphine (2.20 g, 8.4 mmol), and lithium azide (1.02 g, 21.0 mmol) were suspended in dry DMF and carbon tetrabromide (2.78 g, 8.14 mmol) was added to the suspension. After stirring for 3 h at room temperature, the solvent was evaporated to concentrate. The residue was purified by column chromatography on silica gel with chloroform–methanol (5:1 v/v) to give the title compound (775 mg, 88.0%): δ_H (270 MHz; DMSO-d_6) 3.51–3.72 (2H, m, 5′-H), 3.94–4.27, (2H, m, 3′-H, 4′-H), 4.63 (1H, q, 2′-H), 5.4 (1H, d, 3′-OH), 5.63 (1H, d, 2′-OH), 5.9 (1H, d, 1′-H), 8.08 (1H, s, 2-H), 8.33 (1H, s, 8-H). Anal. found: C, 40.95; H, 3.68; N, 33.32. Calcd for $C_{10}H_{11}N_7O_4$: C, 40.96; H, 3.78; N, 33.44; FAB MS. Found *m/z* 294 (M+1), calcd 293.09.

4.1.6. 5′-Amino-5′-deoxy-inosine (5′-NH_2-Ino) (2b). 10% Pd/C (ca. 0.1 g) was added to a solution of 5′-N_3-Ino (239 mg, 1.0 mmol) in methanol–DMF mixed solvent (1:1 v/v) (50 mL). After being stirred for 2 h under a hydrogen atmosphere (1 atm), the mixture was filtered and the filtrate was evaporated to concentrate and a small amount of methanol was added to the solution. Further, ether was added to give the title compound as white powder (245 mg, 92%): δ_H (270 MHz; DMSO-d_6) 2.71–2.91 (2H, m, 5′-H), 3.45 (2H, bs, 5′-NH_2), 3.88, (1H, q, 4′-H), 4.13 (1H, q, 3′-H), 4.57 (1H, t, 2′-H), 5.2–5.4 (2H, bs, 3′-OH, 2′-OH), 5.84 (1H, d, 1′-H), 8.06 (1H, s, 2-H), 8.36 (1H, s, 8-H). Anal. found: C, 44.67; H, 5.11; N, 25.91. Calcd for $C_{10}H_{13}N_5O_4$: C, 44.94; H, 4.90; N, 26.21.); FAB MS. Found *m/z* 268 (M+1), calcd 267.10.

4.1.7. N^{α}-*tert*-Butyloxycarbonyl-N^5-(5′-deoxy-5′-inosyl)-L-isoglutamine (Boc-isoGln(5′I)-OBzl) (3b). To a solution of *N*-*tert*-butyloxycarbonyl-L-glutamic acid γ-benzyl ester (Boc-L-Glu(OBzl)-OH) (1.32 g, 3.90 mmol), HOBt (526 mg, 3.9 mmol), and BOP reagent (1.15 g, 3.9 mmol) in DMF (50 mL), diisopropylethylamine (613 mg, 3.9 mmol) was added. After 30 s, 5′-NH_2-Ino (1.04 g, 3.9 mmol) was added and stirring was continued for 1 h at room temperature. The solvent was removed under a

reduced pressure and the residue was purified by column chromatography on silica gel with chloroform–methanol (5:1 v/v) to give the title compound as white powder (1.56 g, 68.0%): ν_{max} (KBr disc)/cm^{-1} 3420, 1680, 1520, 1460, 1390, 1250, and 1170; δ_H (270 MHz; DMSO-d_6) 1.33 (9H, s, *t*-Bu–H), 1.63–2.00 (2H, m, β-CH$_2$), 2.36 (2H, γ-CH$_2$), 3.24–3.43 (2H, m, 5′-H), 3.76–4.06 (3H, m, 2′-H, 3′-H, 4′-H), 4.53 (1H, q, α-CH$_2$), 5.06 (2H, s, PhCH$_2$), 5.31 (1H, d, 3′-OH), 5.47 (1H, d, 2′-OH), 5.83 (1H, d, 1′-H), 6.98 (1H, d, Boc-NH), 7.27–7.40 (5H, m, Ar-H), 8.00–8.12 (2H, m, 2-H, 5′-NH) 8.30 (1H, s, 8-H). Found: C, 55.01; H, 6.12; N, 14.01. Calcd for $C_{27}H_{34}N_6O_9$: C, 55.28; H, 5.84; N, 14.33; FAB MS. Found *m/z* 587 (M+1), calcd 586.2387.

4.1.8. N^5-(5′-Deoxy-5′-inosyl)-L-isoglutamine benzyl ester trifluoroacetic acid salt (TFA-isoGln(5′I)-OBzl) (4b). Boc-isoGln(5′I)-OBzl (1.01 g, 1.72 mmol) was dissolved in TFA (10 mL) and the solution was kept at 0°C for 30 min. Ether was added to the reaction mixture to give the title compound as an amorphous solid (1.03 g, 99%).

4.1.9. N^{α}-Fluorenylmethyloxycarbonyl-N^5-(5′-deoxy-5′-inosyl)-L-isoglutamine (Fmoc-isoGln(5′I)-OH) (6b). To a solution of TFA·isoGln(5′I)-OBzl (910 mg, 1.51 mmol) and 9-fluorenylmethyl *N*-succinimidyl carbonate (Fmoc-OSu) (611 mg, 1.81 mmol) in DMF (50 mL), diisopropylethylamine (475 mg, 3.02 mmol) was added at 0°C. The reaction mixture was continuously stirred for 10 min at room temperature. The solvent was removed under a reduced pressure and the residue was purified by column chromatography on silica gel with chloroform–methanol (7:1 v/v) to give Fmoc-isoGln(5′I)-OBzl as white powder. The powder was filtered and washed with methanol and dried under a reduced pressure to give Fmoc-isoGln(5′I)-OBzl (943.8 mg, 87.7%). 10% Pd/C (ca. 0.1 g) was added to a solution of Fmoc-isoGln(5′I)-OBzl (943.8 mg, 1.32 mmol) in methanol–DMF (1:1 v/v) (250 mL). After the reaction mixture was stirred for 2 h under a hydrogen atmosphere (1 atm), the catalyst was filtered and the filtrate was evaporated to concentrate and a small amount of methanol was added to the solution. Ether was further added and the precipitate formed was filtered to give 880 mg of the title compound (1.32 mmol, 87.4%): ν_{max} (KBr disc)/cm^{-1} 3300, 3060, 2930, 1700, 1660, 1320, 1260, 1150, and 1090; δ_H (270 MHz; DMSO-d_6) 1.63–1.97 (2H, m, β-CH$_2$), 2.23 (2H, γ-CH$_2$), 3.33–3.46 (2H, m, 5′-H), 3.91–4.07 (3H, m, 4′-H, fluorene–CH$_2$–O), 4.16–4.30 (3H, m, 2′-H, 3′-H, methine of fluorene), 4.53 (1H, t, α-CH), 5.83 (1H, d, 1′-H), 7.26–7.93 (9H, m, fluolene (8H), α-NH), 8.11 (1H, s, 2-H), 8.19 (1H, t, 5′-NH), 8.33 (1H, s, 8-H); HR-FAB MS. Found *m/z* 619.2151 (M+1, error [ppm/mmu, −0.2/−0.1]), calcd 619.2153 (M+H).

4.1.10. N^{α}-*tert*-Butyloxycarbonyl-N^5-(5′-deoxy-5′-adenyl(N^6-benzoyl))-L-isoglutamine Boc-isoGln(5′A(N^6-Bz))-OBzl (3d). To a solution of *N*-*tert*-butyloxycarbonyl-L-glutamic acid-γ-benzyl ester (Boc-L-Glu(OBzl)-OH) (1.40 g, 4.04 mmol), HOBt (1.36 g, 4.04 mmol), and BOP reagent (546 mg, 4.04 mmol) in DMF (100 mL), diisopropylethylamine (653 mg, 4.04 mmol) was added. After 30 s, 5′-NH$_2$-Ado(N^6-Bz)[12] (1.5 g, 4.04 mmol) was added and stirring was continued for 1 h at room temperature. The solvent was removed under a reduced pressure and the residue was purified by column chromatography on silica gel with chloroform–methanol (7:1 v/v) to give the title compound as white powder (2.11 g, 75.8%): ν_{max} (KBr disc)/cm^{-1} 3320, 1680, 1540, 1450, 1380, 1250, and 1170; δ_H (270 MHz; DMSO-d_6) 1.33 (9H, s, *t*-Bu–H), 1.66–2.00 (2H, m, β-CH$_2$), 2.36 (2H, γ-CH$_2$), 3.29–3.54 (2H, m, 5′-H), 3.92–4.13 (3H, m, 2′-H, 3′-H, 4′-H), 4.76 (1H, q, α-CH), 5.06 (2H, s, PhCH$_2$), 5.37 (1H, d, 3′-OH), 5.58 (1H, d, 2′-OH), 6.00 (1H, d, 1′-H), 6.98 (1H, d, Boc-NH), 7.27–7.40 (5H, m, Ar-H), 7.56 (2H, t, Ar-*m*-H), 7.66 (1H, t, Ar-*p*-H), 8.05 (2H, d, Ar-*m*-H), 8.13 (1H, t, 5′-NH), 8.71 (1H, s, 2-H) 8.80 (1H, s, 8-H), 11.25 (1H, s, 4-NH); Found: C, 56.66; H, 5.59; N, 14.22. Calcd for $C_{34}H_{39}N_7O_9$: C, 59.21; H, 5.70; N, 14.22; FAB MS. Found *m/z* 690 (M+1), calcd 689.28.

4.1.11. N^5-(5′-Deoxy-5′-inosyl)-L-isoglutamine benzyl ester trifluoroacetic acid salt TFA·isoGln(5′A(N^6-Bz))-OBzl (4d). Boc-isoGln(5′A(N^6-Bz))-OBzl (2.11 g, 3.03 mmol) was dissolved in TFA (10 mL) and the solution was kept at 0°C for 30 min. Ether (500 mL) was added to the solution to give the title compound as an amorphous solid (1.28 g, 60%).

4.1.12. N^{α}-Fluorenylmethyloxycarbonyl-N^5-(5′-deoxy-5′-adenyl(N^6-benzoyl))-L-isoglutamine (Fmoc-isoGln(5′A(N^6-Bz))-OH) (6d). To a solution of TFA·isoGln(A(N^6-Bz))-OBzl (3.60 g, 5.12 mmol) and 9-fluorenylmethyl *N*-succinimidyl carbonate (Fmoc-OSu) (2.07 g, 6.14 mmol) in DMF (100 mL), diisopropylethylamine (1.32 g, 10.5 mmol) was added at 0°C. The mixture was continuously stirred for 10 min at room temperature. The solvent was removed under a reduced pressure and methanol was added to the residue to precipitate Fmoc-isoGln(A(N^6-Bz))-OBzl as white powder. This powder was filtered and washed with methanol and dried under a reduced pressure (2.20 g, 62.3%). 10% Pd/C (10%, ca. 1 g) was added to a solution of Fmoc-isoGln(A(N^6-Bz))-OBzl (3.30 g, 4.78 mmol) in methanol–DMF mixed solvent (30:70 v/v, 800 mL). After the reaction mixture was stirred for 12 h under a hydrogen atmosphere (1 atm), the catalyst was filtered and the filtrate was evaporated to concentrate and the residue was purified by column chromatography on silica gel with chloroform–methanol (1:1 v/v). The fraction (R_f=0.3 with 1:5 chloroform–methanol) was evaporated to concentrate and the title compound was deposited as powder (1.64 g, 49%): ν_{max} (KBr disc)/cm^{-1} 3300, 3060, 1690, 1550, 1440, 1390, 1290, and 1100; δ_H (270 MHz; DMSO-d_6) 1.73–2.07 (2H, m, β-CH$_2$), 2.38 (2H, γ-CH$_2$), 3.35–3.64 (2H, m, 5′-H), 3.95–4.34 (6H, m, 4′-H, fluorene–CH$_2$–O, 2′-H, 3′-H, methine of fluorene), 4.77 (1H, q, α-CH), 5.38 (1H, d, 3′-OH), 5.62 (1H, d, 2′-OH), 6.02 (1H, d, 1′-H), 7.22–8.05 (14H, m, fluolene (8H), α-NH, Ar (5H)), 8.24 (1H, t, 5′-NH), 8.72 (1H, s, 8-H), 8.80 (1H, s, 2-H), 11.25 (1H, s, 6-NH); HR-FAB MS. Found *m/z* 722.2584 (M+1, error [ppm/mmu, +1.5/+1.1]), calcd 722.2574 (M+H).

4.1.13. *NH$_2$*-CCU-UAC-UAU-CUC-Lys-*OH* (7). ν_{max} (KBr disc)/cm^{-1} 3400, 1660, 1550, 1480, 1400, 1230, 1150, and 1050; δ_H (270 MHz; DMSO-d_6) 1.34 (4H, m, γ-CH$_2$ (Lys) and δ-CH$_2$ (Lys)), 1.76–1.90 (24H, m, β-CH$_2$ (Glu)), 2.14 (24H, m, γ-CH$_2$ (Glu)), 2.99 (2H, m, ϵ-CH$_2$ (Lys)), 3.33–3.46 (24H, m, 5′-H (U, C and A)), 3.91–4.07

(12H, m, 4′-H (U, C and A)), 4.16–4.30 (24H, m, 2′-H, 3′-H (U, C and A)), 4.53 (13H, m, α-CH (U, C, A and Lys)), 5.17 (12H, m, 3′-OH (U, C and A)), 5.40–5.52 (12H, m, 2′-OH (U, C and A)), 5.64 (5H, m, 5-H (U)), 5.72–5.83 (12H, m, 1′-H (U, C and A)), 7.23 (2H, bs, ε-NH_2 (Lys)), 7.32 (5H, m, 5-H (C)), 7.64 (5H, m, 6-H (U)), 7.92–8.13 (14H, m, α-NH and NH_2), 8.11 (2H, s, 2-H(A)), 8.19–8.22 (17H, m, 5′-NH (U, C and A) and 6-H (C)), 8.33 (2H, s, 8-H(A)), 11.33 (12H, m, 3-NH (U), 4-NH (C), 6-NH (A)); MALDI-TOF MS (α-CHCA). Found *m/z* 4436.51 (M), calcd 4436.65.

4.1.14. *NH*$_2$-III-CCI-CII-CCC-Lys-*OH* (8). ν_{max} (KBr disc)/cm^{-1} 3420, 1660, 1560, 1490, 1390, 1260, 1170, and 1050; δ_H (270 MHz; DMSO-d_6) 1.34 (4H, m, γ-CH_2 (Lys) and δ-CH_2 (Lys)), 1.75–1.92 (24H, m, β-CH_2 (Glu)), 2.11 (24H, m, γ-CH_2 (Glu)), 2.99 (2H, m, ε-CH_2 (Lys)), 3.32–3.47 (24H, m, 5′-H (C and I), 3.91–4.07 (12H, m, 4′-H (C and I)), 4.16–4.34 (24H, m, 2′-H, 3′-H (C and I)), 4.54 (13H, m, α-CH (C, I and Lys)), 5.17 (12H, m, 3′-OH (C and I)), 5.40–5.52 (12H, m, 2′-OH (C and I)), 5.72–5.83 (12H, m, 1′-H (C and I)), 7.23 (2H, bs, ε-NH_2 (Lys)), 7.32 (5H, m, 5-H (C)), 7.92–8.13 (14H, m, α-NH and NH_2), 8.19 (2H, s, 2-H(I)), 8.20–8.25 (17H, m, 5′-NH (C and I) and 6-H (C)), 8.33 (2H, s, 8-H(I)), 11.35 (6H, m, 4-NH (C)); MALDI-TOF MS (α-CHCA). Found *m/z* 4535.15 (M+1), calcd 4533.68.

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References

1. (a) Lander, E. S.; Linton, L. M.; Birren, B.; Nusbaum, C.; Zody, M. C.; Baldwin, J.; Devon, K.; Dewar, K.; Doyle, M.; FitzHugh, W.; Funke, R.; Gage, D.; Harris, K.; Heaford, A.; Howland, J.; Kann, L.; Lehoczky, J.; LeVine, R.; McEwan, P.; McKernan, K.; Meldrim, J.; Mesirov, J. P.; Miranda, C.; Morris, W.; Naylor, J.; Raymond, C.; Rosetti, M.; Santos, R.; Sheridan, A.; Sougnez, C.; Stange-Thomann, N.; Stojanovic, N.; Subramanian, A.; Wyman, D.; Rogers, J.; Sulston, J.; Ainscough, R.; Beck, S.; Bentley, D.; Burton, J.; Clee, C.; Carter, N.; Coulson, A.; Deadman, R.; Deloukas, P.; Dunham, A.; Dunham, I.; Durbin, R.; French, L.; Grafham, D.; Gregory, S.; Hubbard, T.; Humphray, S.; Hunt, A.; Jones, M.; Lloyd, C.; McMurray, A.; Matthews, L.; Mercer, S.; Milne, S.; Mullikin, J. C.; Mungall, A.; Plumb, R.; Ross, M.; Shownkeen, R.; Sims, S.; Waterston, R. H.; Wilson, R. K.; Hillier, L. W.; McPherson, J. D.; Marra, M. A.; Mardis, E. R.; Fulton, L. A.; Chinwalla, A. T.; Pepin, K. H.; Gish, W. R.; Chissoe, S. L.; Wendl, M. C.; Delehaunty, K. D.; Miner, T. L.; Delehaunty, A.; Kramer, J. B.; Cook, L. L.; Fulton, R. S.; Johnson, D. L.; Minx, P. J.; Clifton, S. W.; Hawkins, T.; Branscomb, E.; Predki, P.; Richardson, P.; Wenning, S.; Slezak, T.; Doggett, N.; Cheng, J.F.; Olsen, A.; Lucas, S.; Elkin, C.; Uberbacher, E.; Frazier, M. *Nature* **2001**, *409*, 860–921. (b) Venter, J. C.; Adams, M. D.; Myers, E. W.; Li, P. W.; Mural, R. J.; Sutton, G. G.; Smith, H. O.; Yandell, M.; Evans, C. A.; Holt, R. A.; Gocayne, J. D.; Amanatides, P.; Ballew, R. M.; Huson, D. H.; Wortman, J. R.; Zhang, Q.; Kodira, C. D.; Zheng, X. H.; Chen, L.; Skupski, M.; Subramanian, G.; Thomas, P. D.; Zhang, J.; Gabor Miklos, G. L.; Nelson, C.; Broder, S.; Clark, A. G.; Nadeau, J.; McKusick, V. A.; Zinder, N.; Levine, A. J.; Roberts, R. J.; Simon, M.; Slayman, C.; Hunkapiller, M.; Bolanos, R.; Delcher, A.; Dew, I.; Fasulo, D.; Flanigan, M.; Florea, L.; Halpern, A.; Hannenhalli, S.; Kravitz, S.; Levy, S.; Mobarry, C.; Reinert, K.; Remington, K.; Abu-Threideh, J.; Beasley, E.; Biddick, K.; Bonazzi, V.; Brandon, R.; Cargill, M.; Chandramouliswaran, I.; Charlab, R.; Chaturvedi, K.; Deng, Z.; Di Francesco, V.; Dunn, P.; Eilbeck, K.; Evangelista, C.; Gabrielian, A. E.; Gan, W.; Ge, W.; Gong, F.; Gu, Z.; Guan, P.; Heiman, T. J.; Higgins, M. E.; Ji, R. R.; Ke, Z.; Ketchum, K. A.; Lai, Z.; Lei, Y.; Li, Z.; Li, J.; Liang, Y.; Lin, X.; Lu, F.; Merkulov, G. V.; Milshina, N.; Moore, H. M.; Naik, A. K.; Narayan, V. A.; Neelam, B.; Nusskern, D.; Rusch, D. B.; Salzberg, S.; Shao, W.; Shue, B.; Sun, J.; Wang, Z.; Wang, A.; Wang, X.; Wang, J.; Wei, M.; Wides, R.; Xiao, C.; Yan, C. *Science* **2001**, *291*, 1304–1351.
2. Milligan, J. F.; Matteucci, M. D.; Martin, J. C. *J. Med. Chem.* **1993**, *36*, 1923–1937.
3. Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497–1500. Nielsen, P. E. *Acc. Chem. Res.* **1999**, *32*, 624–630.
4. Wada, T.; Minamimoto, N.; Inaki, Y.; Inoue, Y. *J. Am. Chem. Soc.* **2000**, *122*, 6900–6910.
5. Fields, G. B.; Noble, R. L. *Int. J. Pept. Protein Res.* **1990**, *35*, 161–214.
6. Watanabe, K. A.; Fox, J. J. *Angew. Chem. Int. Ed. Engl.* **1966**, *5*, 579–580.
7. Wada, T.; Minamimoto, N.; Inaki, Y.; Inoue, Y. *Chem. Lett.* **1998**, 1025–1026.
8. Cavalieri, E. L.; Vauthier, E. C.; Cosse-Barbi, A.; Fliszar, S. *Theor. Chem. Acc.* **2000**, *104*, 235–239. Shapiro, R. *NATO Adv. Study Inst. Ser., Ser. A: Life Sci.* **1981**, *40*, 3–18.
9. Duschinsky, R.; Eppenberger, U. *Tetrahedron Lett.* **1967**, 5103–5108. Sowa, T.; Tsunoda, K. *Bull. Chem. Soc. Jpn* **1975**, *48*, 3243–3245.
10. Aimoto, S. *Curr. Org. Chem.* **2001**, *5*, 45–87. Flegel, M.; Johnson, T.; Sheppard, R. C. *Innovation Perspect. Solid Phase Synth. Collect. Pap., Int. Symp., 1st* **1990**, 307–324. King, D. S.; Fields, C. G.; Fields, G. B. *Int. J. Pept. Protein Res.* **1990**, *36*, 255–266.
11. Mihara, H.; Chmielewski, J. A.; Kaiser, E. T. *J. Org. Chem.* **1993**, *58*, 2209–2215.
12. Hata, T.; Yamamoto, I.; Sekine, M. *Chem. Lett.* **1975**, 977–980.
13. Bagley, C. J.; Otteson, K. M.; May, B. L.; McCurdy, S. N.; Pierce, L.; Ballard, F. J.; Wallace, J. C. *Int. J. Pept. Protein Res.* **1990**, *36*, 356–361.