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T7 RNA Polymerase Activation and Improvement of the Transcriptional Sequencing by Polyamines

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Abstract—We examined the possibility to improve the effectiveness of the in vitro transcription system using T7 RNA polymerase by coexistence with organic bases. The effect of the additives was evaluated by measuring the amount of RNA products in comparison with that of the control system (without additive). We found that four commercial bases and a series of ethylated polyamine analogues newly designed were active enhancers in the following activation order, 1,8-bis(ethylamino)octane > 1,8-octanediamine > 1,5-bis(ethylamino)-pentane > cadaverine > 1,8-bis(ethylamino)-4-azaoctane > spermidine 1,18-bis(ethylamino)-5,14-diazaoctadecane > agmatine. It was shown that RNA products were corresponding, only in the presence of active enhancers, to the full length size of the template DNA, and that sequencing signals were enhanced by the presence of active enhancers with high fidelity so that the transcriptional sequencing was further refined to be a highly sensitive sequencing method from a small amount of linear dsDNA. These results suggest that T7 RNA polymerase was activated by the specific binding of the polyamine additive to produce RNA transcripts with fidelity to the template DNA. Therefore, it is expected that the transcriptional sequencing in the presence of active enhancers might be a powerful and sensitive method, in place of the prevalent DNA amplification method, for genomic science projects and clinical and practical gene diagnoses.

Introduction

The rapid and precise production of single stranded RNA transcripts of defined length from DNA templates using bacteriophage promoters and purified RNA polymerase (RNAP) has been, and continues to be, of fundamental importance for research on post-transcriptional RNA modifications, ribozyme, and eukaryotic translation mechanism. Moreover, this technology is now widely used to prepare RNA templates for in vitro translation and to generate highly sensitive hybridization probes for Southern and Northern analyses, RNase protection mapping, in situ hybridization, and cDNA libraries in the genome projects. The usual method of in vitro transcription is to use a bacteriophage RNA polymerase to transcribe the template DNA inserted downstream from either SP6, T7, or T3 phage promoter.[1–3]

Several plasmid vectors containing multiple cloning sites located between two such promoters are commercially available. By choosing the appropriate phage RNAP one can produce large amounts of RNA, complementary to either strand of the template DNA. However, T7 RNAP shows some intrinsic limitations which can lead to low transcription levels despite the presence of a strong promoter.[2,4–7]

PCR method[8,9] is employed as the fundamental amplification procedure of genomic DNA and cDNA in many fields of molecular biology. In the direct sequencing of the PCR product from genomic DNA and cDNA,[10] however, there have been pointed out the problems inherent to the cycle sequencing method; intervention of the accurate sequencing reaction by remained 2′-deoxyribonucleotide 5′-triphosphates (2′-dNTPs) and PCR

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primers.[11] These two are presently inactivated by use of exonuclease I and shrimp alkaline phosphatase. But these enzymes are very expensive and the enzymatic reactions are time consuming. These disadvantages prevent the highthroughput production of sequence data in the projects of genome and cDNA sequence analysis.

We have recently developed a new RNAP-based sequencing method called 'transcriptional sequencing',[12,13] which is characterized by a rapid isothermal sequencing reaction by use of mutated T7 RNAP with an improved incorporation rate of 3′-dNTPs as sequencing terminators. This procedure can help to overcome serious problems in the direct sequencing reaction of genomic DNA simply because, in the transcriptional sequencing, RNAP recognizes and polymerizes only ribonucleotide triphosphates (rNTPs) along the template double stranded (ds) DNA.[13] Since the stability and amounts of RNA transcripts can be affected by the elongation rate, we have faced requisition for further refinement of the transcription reaction with respect to rapid, accurate, and long elongation of RNA for the purpose of the establishment of the advanced micro-sequencing technology. We have supposed that this requirement would be achieved by improvement of the in vitro transcription system.

It is well-known that the in vitro transcription system by use of T7 RNAP is specifically affected by the presence of such additives as inhibitory antibiotics[14] and stimulatory natural polyamines, particularly spermidine,[2,3] and that mouse and human RNA polymerases are stimulated significantly by natural polyamines.[15,16] In addition, it has been recently reported that several synthetic polyamines exert a concentration-dependent enhancement effect on T7 RNAP reaction.[7] These reports suggest that appropriate polyamines could improve the in vitro transcription reaction to produce rapidly and precisely large amounts of RNA transcripts. Thus, we started the wide range of scrutiny examination to find stimulatory agents for the T7 RNAP reaction.

Since there has been no logical strategy, we examined randomly the additive effects of amine-bases with various structure on the in vitro transcription system. Then the results were fed back to further systematic study by a series of acyclic polyamine analogues. In this article we describe the evaluation results of the effect of the polyamine additives on T7 RNAP, key structural factors necessary for the activation of T7 RNAP reaction, the fidelity of RNA transcripts, and the remarkable improvement of the transcriptional sequencing by use of stimulatory polyamine analogues.

Results and Discussion

Increase in the transcriptional activity of T7 RNAP by various polyamines

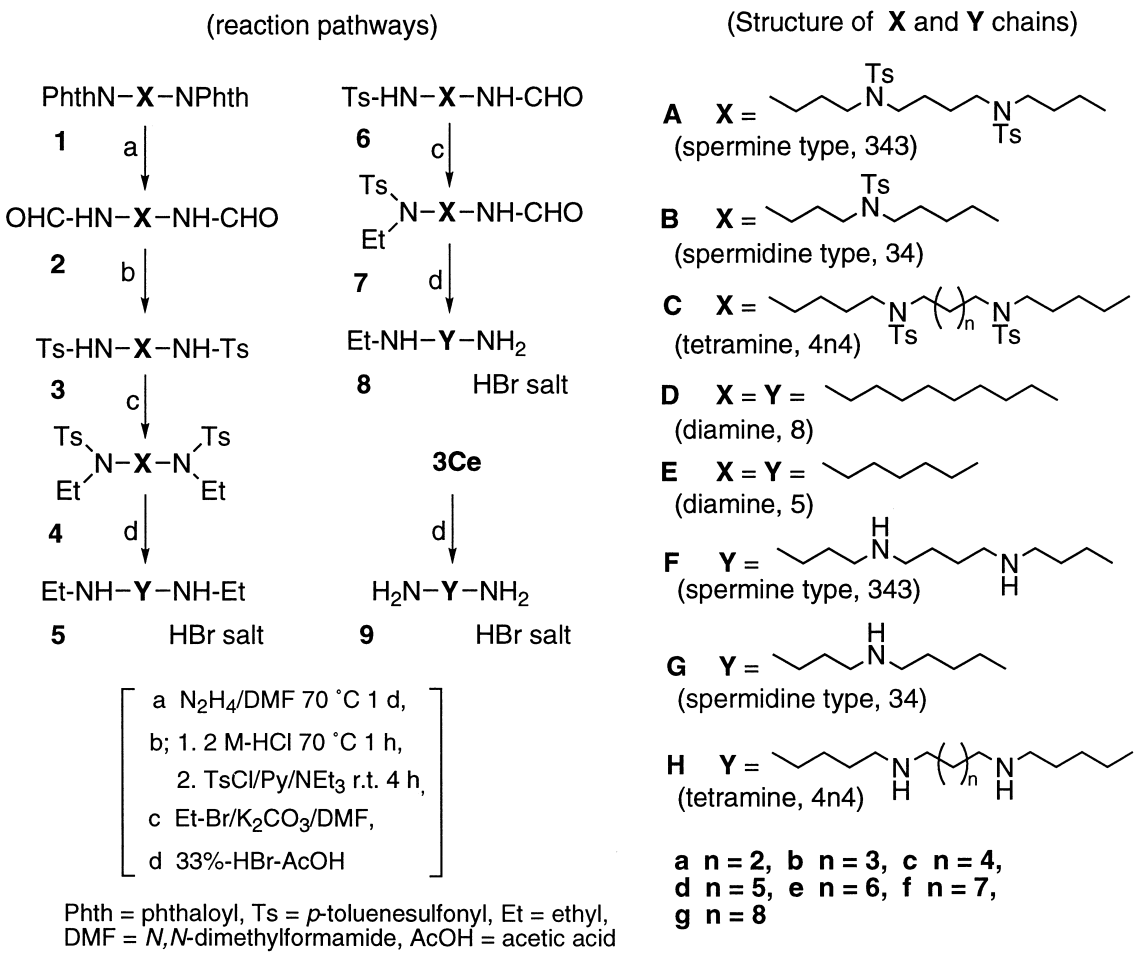
Since there was no intuitive leading principle to activate the T7 RNAP reaction at all, we tested the effects of many organic bases on the in vitro transcription system. As mentioned in Materials and Methods, the transcription reaction utilized for the screening test was carried out at 37 °C for 1 h in a mixture containing Tris-Cl, $MgCl_2$, DTT, GMP, each of rNTP, [α-^{32}P]UTP, and wild type T7 RNAP in the presence of organic base of interest and the linearized template dsDNA, which was derived from pBluescript KS(+) plasmid by PvuII restriction enzyme. Amount of T7 promoter-dependent RNA transcripts produced on the template DNA was measured by electrophoresis followed by autoradiography. Relative effect of the additive organic base was indicated by 'activity index', which is the quotient of the intensity magnitude of the measured radioactivity of RNA transcripts in the presence of the additive divided by that of the control transcription assay (in the absence of the additive). Organic bases, which enhanced the transcription activity of T7 RNAP, and activity indices are summarized in Table 1.

Among many commercially available organic bases examined over 40 samples, only four simple acyclic polyamines, agmatine, cadaverine (1,5-pentanediamine), spermidine, and 1,8-octanediamine, were found to be effective enhancers of the RNAP transcription reaction. The effectiveness was in the order: 1,8-octanediamine > 1,5-pentanediamine > spermidine > agmatine. In contrast, all of newly synthesized polyamine analogues, which were synthesized according to the pathways illustrated in Scheme 1 and characterized by the typical feature of their structures with ethylated terminal amino groups, were found to be, more or less, effective activators. Among them, BET-8 (**5D**), BET-5 (**5E**), BET-43 (**5G**), and BET-484 (**5He**) were the intensive top four in this order. In total, it was shown that the activation order was BET-8 > 1,8-octanediamine > BET-5 > cadaverine > BET-43 > spermidine BET-484 > agmatine. It is obvious that ethylation at the terminal amino group increases the activation

Table 1. Effect of the additive on the transcription reaction of T7 RNA polymerase[a]

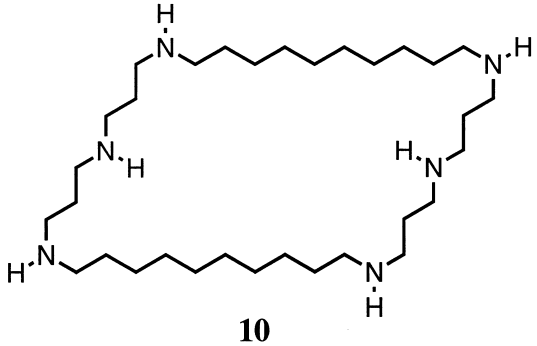
Additive	Characterization	Activity index[b]
5F	BET-343/HBr	1.01
5G	BET-43/HBr	3.05
5Ha	BET-444/HBr	1.21
5Hb	BET-454/HBr	1.18
5Hc	BET-464/HBr	1.12
5Hd	BET-474/HBr	1.29
5He	BET-484/HBr	2.52
5Hf	BET-494/HBr	1.18
5Hg	BET-4104/HBr	2.01
8G	ET34/HBr	1.12
9He	484/HBr	1.41
4D	BET-8/Ts	1.11
5D	BET-8/HBr	4.91
4E	BET-5/Ts	1.43
5E	BET-5/HBr	3.52
1,8-Octanediamine	8	4.62
1,5-Pentanediamine	5	3.40
Spermidine	34	2.58
Agmatine	(Gua)4	2.11

[a]See Experimental for procedure of the transcription reaction and the evaluation method.
[b]The numerical index of the magnitude of the measured radioactivity in the presence of the additive divided by that of the control transcription assay (in the absence of the additive polyamine).



Scheme 1. Synthesis of polyamine Hbr salts.

potential more than the original amine form itself. Macromonocyclic polyamine (**10**), which has been reported to be an intensive enhancer for T7 RNAP,[7] was tested by the present transcription assay system. The result, however, indicated the activity index close to and not more than spermidine (data not shown). These results indicate that we found highly potent activators of T7 RNAP other than spermidine and **10**.



10

In structural viewpoints of active polyamines, enhancers are structure-specific and the enhacing effectiveness is highly dependent on the distance between two terminal amino groups rather than on content of the amino groups. Several enhancing and suppressing structural factors are obviously demonstrated: firstly, the central methylene-chain length seems to govern the structural specificity of the polyamine analogues as the enhancing effector, optimized by penta-, octa-, and deca-methylene chains as the most effective length (refer to pentandiamine, spermidine, octanediamine, and **5Ha–g**). In all cases, the ethyl-substituent plays an auxiliary positive role for the increment of the enhancing effect. The tosyl group, in contrast, exerts the intensive suppressing effect for the reaction (refer to **4D** and **5D**, **4E** and **5E**). Peripheral tetra-methylene chains afford the minor suppressing effect (refer to a set of structural changes for all). The function of tri-methylene chains are skeptical; these exert the enhancing effect in the case of spermidine referred to agmatine, but the intensive suppressing effect in **5F** referred to **5G**. In addition, ethylation at tri-methylene chain markedly decreases the enhancing effectiveness (refer to spermidine and **8G**). With respect to relationship between the basicity of amino group and the intensity of the additive effect, substitution of the primary amino group by the electron donating group like an ethyl substituent is likely to increase the enhancing effect in most cases. In contrast, substitution of the primary amino group by the electron withdrawing group like a tosyl substituent results in rapid decrease in the enhancing effect.

Figure 1 exemplifies the electrophoretic result of a typical case of the present transcription assay using 1,8-octanediamine (lane 2), BET-454 (**5Hb**, lane 3), cadaverine

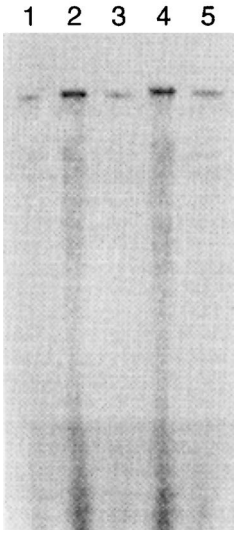


Figure 1. Electrophoretic display of the effect of polyamine additives on the transcription by T7 RNA polymerase: each lane indicates RNA products obtained in the absence of additive (lane 1), or in the presence of 1,8-octanediamine (lane 2), 1,15-bis(ethylamino)-5,11-diazapentadecane (BET-454, lane 3), cadaverine (lane 4), and spermidine (lane 5).

(lane 4), and spermidine (lane 5) as additives in comparison with no additive (lane 1). It is clearly shown that 1,8-octanediamine (activity index 4.62) and cadaverine (3.40) highly increase the amounts of RNA transcripts and spermidine (2.58) is quite active as well. Activation effect by additive on the transcription reaction was concentration-dependent and the best activation was observed in the wide concentration range of 0.2–2.0 mM in most cases (data not shown), consistent with the previous result.[7] It is of special importance in the case of the presence of activator that RNA transcripts grow up till around 300 bp, which correspond to the full length size of the template DNA sequence, without showing any intervention (pausing) of the transcription reaction which is often observed in the chain-growth process affected by the additive acting non-specifically on T7 RNAP. Thus, it is suggested that the polyamine enhancer in the T7 RNAP-mediated transcription should bind to the specific site of T7 RNAP and play a crucial role only for the enhancement of the precise transcription and the elongation of full length RNA transcript.

Transcriptional sequencing in the presence of polyamine additive

Since we have recently developed the transcriptional sequencing method which is characterized by the combination of a rapid isothermal transcriptional sequencing reaction with the effective use of the chain termination reaction,[12,13] it is of our practical interest whether or not the improvement of the transcription system by addition of a polyamine activator will endorse the microanalysis of DNA sequences for which the sensitive and precise transcription must be inevitably achieved. For the examination of this possibility, the transcriptional sequencing was carried out by use of extremely low concentration (10 ng) of the template DNA in the absence of any additive (set A) or in the presence of 1,8-octanediamine as the intensive transcription activator (set B). The results are shown in Fig. 2. Panel A indicates sequencing signals of the set A, and panel B, sequencing signals of the set B.

In the set A, it is obvious that the sequencing signals appear in general with relatively low intensity and that readable sequence is limited to shorter than around 150 bp, including still several unidentifiable very weak signals in this range. Thus, the set A experiment lacks precise sequencing signals and is impracticable for the micro-sequencing of DNA. On the contrary, the set B experiment generates analyzable sequencing signals more than 400 bp, at least, with high fidelity and, thus, is shown to be providing a highly sensitive sequence determination method for micro-amount of the template DNA. These results indicate that the potency of the transcriptional sequencing method was further refined by the coexistence of an active polyamine additive and properly extended to the reliable micro-sequencing method for DNA without any signal drawback.

It is pointed out that there are some intrinsic sequencing problems, mentioned above, in the prevailed DNA amplification sequencing method, which has been used in the world-wide scale as a key sequencing technology in the genome science projects; in addition, for example, the residual template DNA causes problematic electrophoresis in the capillary sequencer and, as a result, the decreased accuracy of the sequencing. Since the present finding supports that an amount of the template DNA can be reduced to a few nanograms without loss of sensitivity and fidelity, the sequencing problems accompanied by the prevailed direct sequencing method will be completely solved by the use of the refined transcriptional sequencing method.[17] Therefore, the transcriptional sequencing in the presence of potent polyamine additive is highly recommended as a powerful and sensitive method for genome science projects and clinical and practical gene diagnoses.

Experimental

General molecular characterization methods

The ^{1}H and ^{13}C NMR spectra were recorded on JEOL GSX-500S (500 MHz) and JNM-GX 400-α FT-NMR spectrometers with Me_4Si as an internal standard in $CDCl_3$ unless otherwise mentioned; chemical shifts (δ) and coupling constants (J) are in parts per million (ppm) and hertz (Hz), respectively. Signal assignments were performed by measurement of ^{1}H–^{1}H COSY and ^{1}H–^{13}C HMBC spectra. IR spectra were obtained on a Shimadzu FT-IR 8100M spectrophotometer. Merck silica gel 60 (Art. 7734, 0.063–0.02) was used for column chromatography, and fragmented Merck precoated silica gel 60 F_{254} plates (Art. 5715, 20×5 cm), for TLC. Product spots on TLC were detected either under a UV-light lamp or in an iodine vapor bath. The uncorrected melting points were measured in a bilayered coverglass (18 m/m, thickness 0.13–0.17 mm) with a micro-melting point apparatus (Yanagimoto Seisakusho, Serial No. 2647). Elemental analyses were performed in the Micro

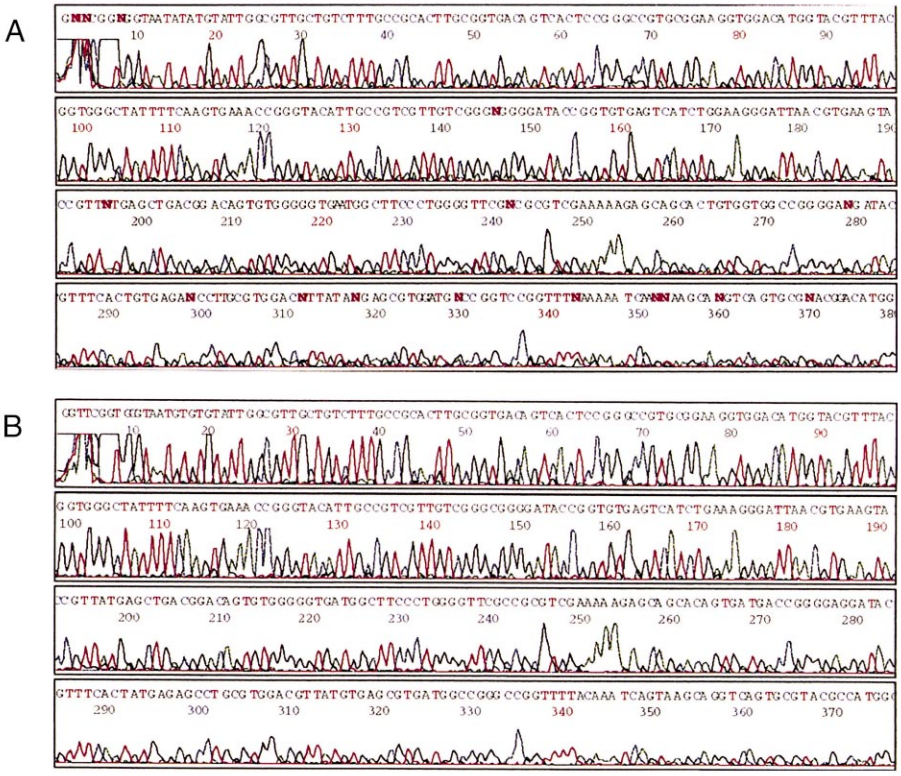


Figure 2. Sequencing signals of the transcriptional sequencings from a small amount of the template DNA: in the absence of any additive (panel A) and in the presence of 1,8-octanediamine (panel B).

Analysis Laboratory of Molecular Characterization in IPCR.

Materials

Agmatine, cadaverine (1,5-pentanediamine), 1,8-octanediamine, and spermidine (1,8-diamino-4-azaoctane) are commercially available (Sigma-Aldrich) and used as additive without further purification. The other polyamine analogues were newly synthesized through the processes shown in detail as follows (see also Scheme 1). Cyclic polyamine [38]N_6C_{10} (**10**) was synthesized by Wako Pure Chemical Co., Ltd.

General synthetic procedure

Purchased 1,*n*-diaminoalkane was tosylated with *p*-toluenesulfonyl chloride in pyridine in the presence of triethylamine to give N^1,N^n-di(*p*-toluenesulfonyl)-1,*n*-diaminoalkane (**M**). The reaction of **M** with alkylating reagents (**N**) was performed under several conditions depending on the structure of the aimed compound. After the connection of **M** and **N**, the terminal groups of the product were treated by the conventional transformation methods[18,19] to give chain-elongated and nitrogen per-tosylated polyamines which were important intermediates for further modification.

1,12-Bis(ethylamino)-4,9-diazadodecane 4HBr (5F). Starting with N^1,N^4-di(*p*-toluenesulfonyl)-1,4-diaminobutane as **M** and *N*-(3-bromopropyl)phthalimide as **N**, N^1,N^4,N^9,N^{12}-tetra(*p*-toluenesulfonyl)-4,9-diaza-1,12-diaminododecane (**3A**) was derived. A mixture of **3A** (0.17 g), potassium carbonate (0.14 g) and bromoethane (57 mg) in 40 mL of DMF was stirred at rt for 3 days and filtered through Celite. The filtrate was evaporated under reduced pressure and the residue was chromatographed on a silica gel column eluted with chloroform: acetone (95:5 v/v). The fraction indicating $R_f = 0.4$ on TLC (chloroform-acetone (95:5 v/v)) was collected and evaporated to dryness to afford N^1,N^4,N^9,N^{12}-tetra(*p*-toluenesulfonyl)-1,12-bis(ethylamino)-4,9-diazadodecane (**4A**, 0.11 g, viscous liquid, 58% yield). Anal. calcd for $C_{42}H_{58}N_4O_8S_4$: C, 57.64; H, 6.68; N, 6.40. Found: C, 57.46; H, 6.60; N, 6.39. IR(KRS): ν 1350 and 1550 cm^{-1} (SO_2). ^1H NMR data: δ 1.07 (t, $J = 7.32$ Hz, Et), 3.19 (quar, $J = 7.32$ Hz, Et), 1.86 (quin, $J = 7.32$ Hz, CH_2 of –$(CH_2)_3$–), 3.11 (t, $J = 7.32$ Hz, CH_2 of –$(CH_2)_3$–), 3.13 (t, $J = 7.32$ Hz, CH_2 of –$(CH_2)_3$–), 1.58 (m, $J = 7.32$ Hz, CH_2 of –$(CH_2)_4$–), 3.10 (quin, $J = 7.32$ Hz, CH_2 of –$(CH_2)_4$–), 2.41 (s, CH_3), 2.42 (s, CH_3), 7.29 (d, $J = 8.30$ Hz), 7.31 (d, $J = 8.30$ Hz), 7.66 (d, $J = 8.30$ Hz), 7.67 (d, $J = 8.30$). ^{13}C NMR data: δ 13.91 (Et), 43.14 (Et), 28.71 (C of –$(CH_2)_3$–), 45.46 (C of –$(CH_2)_3$–), 46.51 (C of –$(CH_2)_3$–), 25.75 (C of –$(CH_2)_4$–), 48.39 (C of –$(CH_2)_4$–), 21.48 (CH_3), 127.07, 127.12, 129.69, 129.77, 136.20, 136.65, 143.16, 143.31. A mixture of **4A** (0.10 g) and phenol (0.21 g) in 33%-HBr AcOH (10 mL) was heated at 75 °C for 20 h and evaporated to dryness under reduced pressure. Dark brown residue was washed several times until colourless with ether at first and then with ether–methanol mixed solvent to give **5F** as colourless powder (87% yield).

1,8-Bis(ethylamino)-4-azaoctane 3HBr (5G). Starting with *N*-(N^3-*p*-toluenesulfonyl-3-aminopropyl)phthalimide as **M** and *N*-(4-bromobutyl)phthalimide as **N**, N^1,N^4,N^8-tri(*p*-toluenesulfonyl)-1,8-diamino-4-azaoctane (**3B**) was derived. A mixture of **3B** (0.16 g), potassium carbonate (0.18 g) and bromoethane (48 μL) in 40 mL of DMF was stirred at rt for 3 days and filtered through Celite. The filtrate was evaporated under reduced pressure and the residue was chromatographed on a silica gel column eluted with chloroform:acetone (95:5 v/v). The fraction indicating R_f = 0.4 on TLC (chloroform:acetone (95:5 v/v)) was collected and evaporated to dryness to afford N^1,N^4,N^8-tri(*p*-toluenesulfonyl)-1,8-bis(ethylamino)-4-azaoctane (**4B**, 0.11 g, viscous liquid, 65% yield). Anal. calcd for $C_{32}H_{45}N_3O_6S_3$: C, 57.89; H, 6.83; N, 6.33. Found: C, 57.61; H, 6.88; N, 6.32. IR(KRS): ν 1350 and 1550 cm^{-1} (SO_2). ^1H NMR data: δ 1.08 (t, J = 7.32 Hz, Et), 1.09 (t, J = 7.32 Hz, Et), 3.18 (quar, J = 7.32 Hz, Et), 3.21 (quar, J = 7.32 Hz, Et), 1.87 (quin, J = 7.32 Hz, CH_2 of –$(CH_2)_3$–), 3.13 (t, J = 7.32 Hz, CH_2 of –$(CH_2)_3$–), 3.15 (t, J = 7.32 Hz, CH_2 of –$(CH_2)_3$–), 1.57 (m, J = 7.32 Hz, CH_2 of –$(CH_2)_4$–), 3.12 (t, J = 7.32 Hz, CH_2 of –$(CH_2)_4$–), 3.12 (t, J = 7.32 Hz, CH_2 of –$(CH_2)_4$–), 2.42 –(s, CH_3), 2.43 (s, CH_3), 7.29 (d, J = 8.30 Hz), 7.31 (d, J = 8.30 Hz), 7.67 (d, J = 8.30 Hz), 7.67 (d, J = 8.30 Hz), 7.68 (d, J = 8.30 Hz). ^{13}C NMR data: δ 13.91 (Et), 14.00 (Et), 42.84 (Et), 43.12 (Et), 28.69 (C of –$(CH_2)_3$–), 46.91 (C of –$(CH_2)_3$–), 48.40 (C of –$(CH_2)_3$–), 25.65 (C of –$(CH_2)_4$–), 25.75 (C of –$(CH_2)_4$–), 45.44 (C of –$(CH_2)_4$–), 46.43 (C of –$(CH_2)_4$–), 21.47 (CH_3), 127.04, 127.07, 127.12, 129.65, 129.69, 129.75, 136.27, 136.66, 136.98, 143.05, 143.18, 143.31. A mixture of **4B** (0.10 g) and phenol (0.28 g) in 33%-HBr AcOH (10 mL) was heated at 75 °C for 20 h and evaporated to dryness under reduced pressure. Dark brown residue was washed several times with ether until colourless and then with ether–methanol mixed solvent to give **5G** as colourless powder (91% yield).

1,14-Bis(ethylamino)-5,10-diazatetradecane 4HBr (5Ha). Starting with N^1,N^4-di(*p*-toluenesulfonyl)-1,4-diaminobutane as **M** and *N*-(4-bromobutyl)phthalimide as **N**, N^1,N^5,N^{10},N^{14}-tetra(*p*-toluenesulfonyl)-1,14-diamino-5,10-diazatetradecane (**3Ca**) was derived. A mixture of **3Ca** (0.18 g), potassium carbonate (0.15 g) and bromoethane (40 μL) in 40 mL of DMF was stirred at rt for 3 days and filtered through Celite. The filtrate was evaporated under reduced pressure and the residue was chromatographed on a silica gel column eluted with chloroform-acetone (95:5 v/v). The fraction indicating R_f = 0.4 on TLC (chloroform:acetone (95:5 v/v)) was collected and evaporated to dryness to afford N^1,N^5,N^{10},N^{14}-tetra(*p*-toluenesulfonyl)-1,14-bis(ethylamino)-5,10-diazatetradecane (**4C**, 0.22 g, viscous liquid, 88% yield). Anal. calcd for $C_{44}H_{62}N_4O_8S_4$: C, 58.51; H, 6.92; N, 6.20. Found: C, 58.43; H, 6.89; N, 6.16. IR(KRS): ν 1350 and 1550 cm^{-1} (SO_2). ^1H NMR data: δ 1.05 (t, J = 7.32 Hz, Et), 3.17 (quar, J = 7.32 Hz, Et), 1.55–1.57 (m, CH_2 of terminal –$(CH_2)_4$–), 3.11–3.13 (m, CH_2 of terminal –$(CH_2)_4$–), 1.56 (m, CH_2 of central–$(CH_2)_4$–), 3.10–3.13 (m, CH_2 of central –$(CH_2)_4$–), 2.41 (s, CH_3), 2.42 (s, CH_3), 7.28 (d, J = 8.30 Hz), 7.30 (d, J = 8.30 Hz), 7.67 (d, J = 8.30 Hz), 7.68 (d, J = 8.30 Hz). ^{13}C NMR data: δ 13.96 (Et), 42.78 (Et), 25.73 (C of terminal –$(CH_2)_4$–), 25.79 (C of terminal –$(CH_2)_4$–), 46.94 (C of terminal –$(CH_2)_4$–), 25.94 (C of central –$(CH_2)_4$–), 48.02 (C of central –$(CH_2)_4$–), 21.48 (CH_3), 127.04, 127.09, 129.64, 129.70, 136.20, 136.63, 137.01, 143.03, 143.18. A mixture of **4C** (0.11 g) and phenol (0.24 g) in 33%-HBr AcOH (10 mL) was heated at 75 °C for 20 h and evaporated to dryness under reduced pressure. Dark brown residue was washed several times with ether until colourless and then with ether–methanol mixed solvent to give **5Ha** as colourless powder (91% yield).

1,15-Bis(ethylamino)-5,11-diazapentadecane 4HBr (5Hb). Starting with N^1,N^5-di(*p*-toluenesulfonyl)-1,5-diaminopentane as **M** and *N*-(4-bromobutyl)phthalimide as **N**, N^1,N^5,N^{11},N^{15}-tetra(*p*-toluenesulfonyl)-1,15-diamino-5,11-diazapentadecane (**3Cb**) was derived. A mixture of **3Cb** (0.19 g), potassium carbonate (0.15 g) and bromoethane (40 μL) in 40 mL of DMF was stirred at rt for 3 days and filtered through Celite. The filtrate was evaporated under reduced pressure and the residue was chromatographed on a silica gel column eluted with chloroform:acetone (95:5 v:v). The fraction indicating R_f = 0.4 on TLC (chloroform:acetone (95:5 v/v)) was collected and evaporated to dryness to afford N^1,N^5,N^{11},N^{15}-tetra(*p*-toluenesulfonyl)-1,15-bis(ethylamino)-5,11-diazapentadecane (**4Cb**, 0.17 g, viscous liquid, 87% yield). Anal. calcd for $C_{45}H_{64}N_4O_8S_4$: C, 58.92; H, 7.03; N, 6.11. Found: C, 58.75; H, 6.97; N, 6.08. IR(KRS): ν 1350 and 1550 cm^{-1} (SO_2). ^1H NMR data: δ 1.05 (t, J = 7.32 Hz, Et), 3.17 (quar, J = 7.32 Hz, Et), 1.55- 1.57 (m, CH_2 of terminal –$(CH_2)_4$–), 3.11- 3.13 (m, CH_2 of terminal –$(CH_2)_4$–), 1.26 (quin, J = 7.32 Hz, CH_2 of central –$(CH_2)_5$–), 1.50 (quin, J = 7.32 Hz, CH_2 of central –$(CH_2)_5$–), 3.06 (t, J = 7.32 Hz, CH_2 of central –$(CH_2)_5$–), 2.41 (s, CH_3), 2.42 (s, CH_3), 7.28 (d, J = 8.30 Hz), 7.30 (d, J = 8.30 Hz), 7.67 (d, J = 8.30 Hz), 7.68 (d, J = 8.30 Hz). ^{13}C NMR data: δ 13.98 (Et), 42.79 (Et), 25.73 (C of terminal –$(CH_2)_4$–), 25.79 (C of terminal –$(CH_2)_4$–), 46.97 (C of terminal –$(CH_2)_4$–), 47.96 (C of terminal –$(CH_2)_4$–), 23.80 (C of central –$(CH_2)_5$–), 28.41 (C of central –$(CH_2)_5$–), 48.42 (C of central –$(CH_2)_5$–), 21.47 (CH_3), 127.02, 127.07, 129.64, 129.67, 136.68, 136.99, 143.03, 143.13. A mixture of **4Cb** (0.16 g) and phenol (0.33 g) in 33%-HBr AcOH (13 mL) was heated at 75 °C for 20 h and evaporated to dryness under reduced pressure. Dark brown residue was washed several times with ether until colourless and then with ether-methanol mixed solvent to give **5Hb** as colourless powder (88% yield).

1,16-Bis(ethylamino)-5,12-diazahexadecane 4HBr (5Hc). Starting with N^1,N^6-di(*p*-toluenesulfonyl)-1,6-diaminohexane as **M** and *N*-(4-bromobutyl)phthalimide as **N**, N^1,N^5,N^{12},N^{16}-tetra(*p*-toluenesulfonyl)-1,16-diamino-5,12-diazahexadecane (**3Cc**) was derived. A mixture of **3Cc** (0.21 g), potassium carbonate (0.16 g) and bromo-ethane (44 μL) in 40 mL of DMF was stirred at rt for 3 days and filtered through Celite. The filtrate was evaporated under reduced pressure and the residue was chromatographed on a silica gel column eluted with chloroform:acetone (95:5 v/v). The fraction indicating R_f = 0.4 on TLC (chloroform:acetone (95:5 v/v)) was collected and evaporated to dryness to afford N^1,N^5,N^{12},N^{16}-tetra(*p*-toluenesulfonyl)-1,16-bis(ethylamino)-5,12-diazahexa-decane

(**4Cc**, 0.24 g, viscous liquid, 99% yield). Anal. calcd for $C_{46}H_{66}N_4O_8S_4$: C, 59.33; H, 7.14; N, 6.02. Found: C, 59.24; H, 7.07; N, 5.98. IR(KRS): ν 1350 and 1550 cm^{-1} (SO_2). ^1H NMR data: δ 1.06 (t, $J=7.32$ Hz, Et), 3.17 (quar, $J=7.32$ Hz, Et), 1.57–1.58 (m, CH_2 of terminal –$(CH_2)_4$–), 3.11 (t, $J=7.32$ Hz, CH_2 of terminal –$(CH_2)_4$–), 3.12 (t, $J=7.32$ Hz, CH_2 of terminal –$(CH_2)_4$–), 1.26 (quin, $J=7.32$ Hz, CH_2 of central–$(CH_2)_6$–), 1.50 (quin, $J=7.32$ Hz, CH_2 of central –$(CH_2)_6$–), 3.06 (t, $J=7.32$ Hz, CH_2 of central –$(CH_2)_6$–), 2.41 (s, CH_3), 2.42 (s, CH_3), 7.28 (d, $J=8.30$ Hz), 7.30 (d, $J=8.30$ Hz), 7.67 (d, $J=8.30$ Hz), 7.68 (d, $J=8.30$ Hz). ^{13}C NMR data: δ 14.00 (Et), 42.79 (Et), 25.75 (C of terminal –$(CH_2)_4$–), 25.79 (C of terminal –$(CH_2)_4$–), 46.99 (C of terminal –$(CH_2)_4$–), 47.83 (C of terminal –$(CH_2)_4$–), 26.30 (C of central–$(CH_2)_6$–), 28.71 (C of central–$(CH_2)_6$–), 48.42 (C of central –$(CH_2)_6$–), 21.47 (CH_3), 127.04, 127.07, 129.65, 136.76, 137.01, 143.03, 143.10. A mixture of **4Cc** (0.16 g) and phenol (0.33 g) in 33%-HBr AcOH (13 mL) was heated at 75 °C for 20 h and evaporated to dryness under reduced pressure. Dark brown residue was washed several times with ether until colourless and then with ether-methanol mixed solvent to give **5Hc** as colourless powder (89% yield).

1,17-Bis(ethylamino)-5,13-diazaheptadecane 4HBr (5Hd). Starting with N^1,N^7-di(*p*-toluenesulfonyl)-1,7-diaminoheptane as **M** and *N*-(4-bromobutyl)phthalimide as **N**, N^1,N^5,N^{13},N^{17}-tetra(*p*-toluenesulfonyl)-1,17-diamino-5,13-diazaheptadecane (**3Cd**) was derived. A mixture of **3Cd** (0.16 g), potassium carbonate (0.18 g) and bromoethane (38 μL) in 40 mL of DMF was stirred at rt for 3 days and filtered through Celite. The filtrate was evaporated under reduced pressure and the residue was chromatographed on a silica gel column eluted with chloroform:acetone (95:5 v/v). The fraction indicating $R_f=0.4$ on TLC (chloroform:acetone (95:5 v/v)) was collected and evaporated to dryness to afford N^1,N^5,N^{13},N^{17}-tetra(*p*-toluenesulfonyl)-1,17-bis(ethylamino)-5,13-diazaheptadecane (**4Cd**, 0.15 g, viscous liquid, 89% yield). Anal. calcd for $C_{47}H_{68}N_4O_8S_4$: C, 59.72; H, 7.25; N, 5.93. Found: C, 59.66; H, 7.27; N, 6.01. IR(KRS): ν 1350 and 1550 cm^{-1} (SO_2). ^1H NMR data: δ 1.07 (t, $J=7.32$ Hz, Et), 3.17 (quar, $J=7.32$ Hz, Et), 1.57–1.58 (m, CH_2 of terminal –$(CH_2)_4$–), 3.11 (t, $J=7.32$ Hz, CH_2 of terminal –$(CH_2)_4$–), 3.13 (t, $J=7.32$ Hz, CH_2 of terminal –$(CH_2)_4$–), 1.23 (quin, $J=7.32$ Hz, CH_2 of central –$(CH_2)_7$–), 1.25 (quin, $J=7.32$ Hz, CH_2 of central –$(CH_2)_7$–), 1.50 (quin, $J=7.32$ Hz, CH_2 of central –$(CH_2)_7$–), 3.06 (t, $J=7.32$ Hz, CH_2 of central –$(CH_2)_7$–), 2.42 (s, CH_3), 7.29 (d, $J=8.30$ Hz), 7.30 (d, $J=8.30$ Hz), 7.67 (d, $J=8.30$ Hz). ^{13}C NMR data: δ 14.00 (Et), 42.78 (Et), 25.78 (C of terminal –$(CH_2)_4$–), 46.99 (C of terminal –$(CH_2)_4$–), 47.80 (C of terminal –$(CH_2)_4$–), 26.63 (C of central –$(CH_2)_7$–), 28.69 (C of central –$(CH_2)_7$–), 28.76 (C of central –$(CH_2)_7$–), 48.50 (C of central –$(CH_2)_7$–), 21.47 (CH_3), 127.04, 127.07, 129.64, 136.80, 137.01, 143.06. A mixture of **4Cd** (0.14 g) and phenol (0.28 g) in 33%-HBr AcOH (13 mL) was heated at 75 °C for 20 h and evaporated to dryness under reduced pressure. Dark brown residue was washed several times with ether until colourless and then with ether-methanol mixed solvent to give **5Hd** as colourless powder (91% yield).

1,18-Bis(ethylamino)-5,14-diazaoctadecane 4HBr (5He). Starting with N^1,N^8-di(*p*-toluenesulfonyl)-1,8-diaminooctane as **M** and *N*-(4-bromobutyl)phthalimide as **N**, N^1,N^5,N^{14},N^{18}-tetra(*p*-toluenesulfonyl)-1,18-diamino-5,14-diazaoctadecane (**3Ce**) was derived. A mixture of **3Ce** (0.18 g), potassium carbonate (0.14 g) and bromoethane (37 μL) in 40 mL of DMF was stirred at rt for 3 days and filtered through Celite. The filtrate was evaporated under reduced pressure and the residue was chromatographed on a silica gel column eluted with chloroform-acetone (95:5 v/v). The fraction indicating $R_f=0.4$ on TLC (chloroform:acetone (95:5 v/v)) was collected and evaporated to dryness to afford N^1,N^5,N^{14},N^{18}-tetra(*p*-toluenesulfonyl)-1,18-bis(ethylamino)-5,14-diazaoctadecane (**4Ce**, 0.17 g, viscous liquid, 92% yield). Anal. calcd for $C_{48}H_{70}N_4O_8S_4$: C, 60.09; H, 7.35; N, 5.84. Found: C, 59.98; H, 7.30; N, 5.85. IR(KRS): ν 1350 and 1550 cm^{-1} (SO_2). ^1H NMR data: δ 1.07 (t, $J=7.32$ Hz, Et), 3.18 (quar, $J=7.32$ Hz, Et), 1.58 (m, CH_2 of terminal –$(CH_2)_4$–), 3.12 (t, $J=7.32$ Hz, CH_2 of terminal –$(CH_2)_4$–), 3.13 (t, $J=7.32$ Hz, CH_2 of terminal –$(CH_2)_4$–), 1.24 (m, CH_2 of central –$(CH_2)_8$–), 1.50 (quin, J = 7.32, CH_2 of central –$(CH_2)_8$–), 3.06 (t, $J=7.32$ Hz, CH_2 of central –$(CH_2)_8$–), 2.42 (s, CH_3), 7.29 (d, $J=8.30$ Hz), 7.29 (d, $J=8.30$ Hz), 7.67 (d, $J=8.30$ Hz). ^{13}C NMR data: δ 14.01 (Et), 42.78 (Et), 25.75 (C of terminal –$(CH_2)_4$–), 25.78 (C of terminal –$(CH_2)_4$–), 46.99 (C of terminal –$(CH_2)_4$–), 47.74 (C of terminal –$(CH_2)_4$–), 26.63 (C of central –$(CH_2)_8$–), 28.72 (C of central –$(CH_2)_8$–), 29.10 (C of central –$(CH_2)_8$–), 48.50 (C of central –$(CH_2)_8$–), 21.47 (CH_3), 127.04, 127.07, 129.62, 136.84, 137.01, 143.03. A mixture of **4Ce** (0.16 g) and phenol (0.32 g) in 33%-HBr AcOH (10 mL) was heated at 75 °C for 20 h and evaporated to dryness under reduced pressure. Dark brown residue was washed several times with ether until colourless and then with ether-methanol mixed solvent to give **5He** as colourless powder (85% yield).

1,19-Bis(ethylamino)-5,15-diazanonadecane 4HBr (5Hf). Starting with N^1,N^9-di(*p*-toluenesulfonyl)-1,9-diaminononane as **M** and *N*-(4-bromobutyl)phthalimide as **N**, N^1,N^5,N^{15},N^{19}-tetra(*p*-toluenesulfonyl)-1,19-diamino-5,15-diazanonadecane (**3Cf**) was derived. A mixture of **3Cf** (0.17 g), potassium carbonate (0.13 g) and bromoethane (34 μL) in 40 mL of DMF was stirred at rt for 3 days and filtered through Celite. The filtrate was evaporated under reduced pressure and the residue was chromatographed on a silica gel column eluted with chloroform:acetone (95:5 v/v). The fraction indicating $R_f=0.4$ on TLC (chloroform:acetone (95:5 v/v) was collected and evaporated to dryness to afford N^1,N^5,N^{15},N^{19}-tetra(*p*-toluenesulfonyl)-1,19-bis(ethylamino)-5,15-diazanonadecane (**4Cf**, 0.17 g, viscous liquid, 98% yield). Anal. calcd for $C_{49}H_{72}N_4O_8S_4$: C, 60.46; H, 7.46; N, 5.76. Found: C, 60.28; H, 7.42; N, 5.74. IR(KRS): ν 1350 and 1550 cm^{-1}(SO_2). ^1H NMR data: δ 1.07 (t, $J=7.32$ Hz, Et), 3.18 (quar, $J=7.32$ Hz, Et), 1.57 (m, CH_2 of terminal –$(CH_2)_4$–), 3.12 (t, $J=7.32$ Hz, CH_2 of terminal –$(CH_2)_4$–), 3.13 (t, $J=7.32$ Hz, CH_2 of terminal –$(CH_2)_4$–), 1.23 (m, CH_2 of central –$(CH_2)_9$–), 1.49 (quin, $J=7.32$ Hz, CH_2 of central –$(CH_2)_9$–), 3.06 (t, $J=7.32$ Hz, CH_2 of central –$(CH_2)_9$–), 2.42 (s, CH_3), 7.29

(d, $J=8.30$ Hz), 7.67 (d, $J=8.30$ Hz). ^{13}C NMR data: δ 14.01 (Et), 42.78 (Et), 25.75 (C of terminal –$(CH_2)_4$–), 25.78 (C of terminal –$(CH_2)_4$–), 46.99 (C of terminal –$(CH_2)_4$–), 47.71 (C of terminal –$(CH_2)_4$–), 26.72 (C of central –$(CH_2)_9$–), 28.74 (C of central –$(CH_2)_9$–), 29.10 (C of central –$(CH_2)_9$–), 29.43 (C of central –$(CH_2)_9$–), 48.50 (C of central –$(CH_2)_9$–), 21.47 (CH_3), 127.04, 127.07, 129.62, 136.84, 137.01, 143.03. A mixture of **4Cf** (0.16 g) and phenol (0.32 g) in 33%-HBr AcOH (10 mL) was heated at 75 °C for 20 h and evaporated to dryness under reduced pressure. Dark brown residue was washed several times with ether until colourless and then with ether-methanol mixed solvent to give **5Hf** as colourless powder (89% yield).

1,20-Bis(ethylamino)-5,16-diazaeicosane 4HBr (5Hg). Starting with N^1,N^{10}-di(*p*-toluenesulfonyl)-1,10-diaminodecane as **M** and *N*-(4-bromobutyl)phthalimide as **N**, N^1,N^5,N^{16},N^{20}-tetra(*p*-toluenesulfonyl)-1,20-diamino-5,16-diazaeicosane (**3Cg**) was derived. A mixture of **3Cg** (0.17 g), potassium carbonate (0.13 g) and bromoethane (34 μL) in 40 mL of DMF was stirred at rt for 3 days and filtered through Celite. The filtrate was evaporated under reduced pressure and the residue was chromatographed on a silica gel column eluted with chloroform:acetone (95:5 v/v). The fraction indicating $R_f=0.4$ on TLC (chloroform:acetone (95:5 v/v)) was collected and evaporated to dryness to afford N^1,N^5,N^{16},N^{20}-tetra(*p*-toluenesulfonyl)-1,20-bis(ethylamino)-5,16-diazaeicosane (**4Cg**, 0.18 g, viscous liquid, 98% yield). Anal. calcd for $C_{50}H_{74}N_4O_8S_4$: C, 60.82; H, 7.55; N, 5.67. Found: C, 60.94; H, 7.59; N, 5.60. IR(KRS): ν 1350 and 1550 cm^{-1} (SO_2). ^{1}H NMR data: δ 1.07 (t, $J=7.32$ Hz, Et), 3.18 (quar, $J=7.32$ Hz, Et), 1.57 (m, CH_2 of terminal –$(CH_2)_4$–), 3.12 (t, $J=7.32$ Hz, CH_2 of terminal –$(CH_2)_4$–), 3.13 (t, $J=7.32$ Hz, CH_2 of terminal –$(CH_2)_4$–), 1.23 (m, CH_2 of central –$(CH_2)_{10}$–), 1.49 (quin, $J=7.32$ Hz, CH_2 of central –$(CH_2)_{10}$–), 3.06 (t, $J=7.32$ Hz, CH_2 of central –$(CH_2)_{10}$–), 2.42 (s, CH_3), 7.29 (d, $J=8.30$ Hz), 7.68 (d, $J=8.30$ Hz). ^{13}C NMR data: δ 14.01 (Et), 42.78 (Et), 25.76 (C of terminal –$(CH_2)_4$–), 46.99 (C of terminal –$(CH_2)_4$–), 47.70 (C of terminal –$(CH_2)_4$–), 26.75 (C of central –$(CH_2)_{10}$–), 28.72 (C of central-$(CH_2)_{10}$-), 29.17 (C of central –$(CH_2)_{10}$–), 29.43 (C of central –$(CH_2)_{10}$–), 48.50 (C of central –$(CH_2)_{10}$–), 21.47 (CH_3), 127.04, 127.07, 129.64, 136.86, 137.03, 143.03. A mixture of **4Cg** (0.17 g) and phenol (0.32 g) in 33%-HBr AcOH (10 mL) was heated at 75 °C for 20 h and evaporated to dryness under reduced pressure. Dark brown residue was washed several times with ether until colourless and then with ether–methanol mixed solvent to give **5Hg** as colourless powder (90% yield).

1-Ethylamino-8-amino-4-azaoctane 3HBr (8G). Starting with *N*-(N^4-*p*-toluenesulfonyl-4-aminobutyl)phthalimide as **M** and *N*-(*p*-toluenesulfonyl)-3-bromopropylamine as **N**, N^1,N^4-di(*p*-toluenesulfonyl)-N^8-formyl-1,8-diamino-4-azaoctane (**6B**) was derived. A mixture of **6B** (0.24 g), potassium carbonate (0.34 g) and bromoethane (90 μL) in 50 mL of DMF was stirred at rt for 3 days and filtered through Celite. The filtrate was evaporated under reduced pressure and the residue was chromatographed on a silica gel column eluted with chloroform:acetone (7:3 v/v). The fraction indicating $R_f=0.3$ on TLC (chloroform:acetone (9:1 v/v)) was collected and evaporated to dryness to afford N^1-ethyl-N^1,N^4-di(*p*-toluenesulfonyl)-N^8-formyl-1,8-diamino-4-azaoctane (**7B**, 0.22 g, viscous liquid, 88% yield). Anal. calcd for $C_{24}H_{35}N_3O_5S_2$: C, 57.56; H, 6.92; N, 8.24. Found: C, 57.57; H, 6.97; N, 7.99. IR(KRS): ν 1680 (CHO), 1350 and 1550 (SO_2) cm^{-1}. ^{1}H NMR data: δ 1.07 (t, $J=7.32$ Hz, Et), 1.09 (t, $J=7.32$ Hz, Et), 3.19 (quar, $J=7.32$ Hz, Et), 1.86 (quin, $J=7.32$ Hz, CH_2 of –$(CH_2)_3$–), 3.14 (t, $J=7.32$ Hz, CH_2 of –$(CH_2)_3$–), 3.18 (t, $J=7.32$ Hz, CH_2 of –$(CH_2)_3$–), 1.60 (quin, $J=7.32$ Hz, CH_2 of –$(CH_2)_4$–), 1.67 (quin, $J=7.32$ Hz, CH_2 of –$(CH_2)_4$–), 3.10 (t, $J=7.32$ Hz, CH_2 of –$(CH_2)_4$–), 3.34 (quar, $J=7.32$ Hz, CH_2 of –$(CH_2)_4$–), 2.43 (s, CH_3), 6.05 (bs, NH), 7.31 (d, $J=8.30$ Hz), 7.66 (d, $J=8.30$ Hz), 7.67 (d, $J=8.30$ Hz), 8.16 (bs, CHO). ^{13}C NMR data: δ 13.91 (Et), 43.40 (Et), 28.23 (C of –$(CH_2)_3$–), 45.54 (C of –$(CH_2)_3$–), 46.92 (C of –$(CH_2)_3$–), 26.39 (C of –$(CH_2)_4$–), 37.36 (C of –$(CH_2)_4$–), 49.08 (C of –$(CH_2)_4$–), 21.48 (CH_3), 127.04, 127.12, 129.77, 135.91, 136.37, 143.39, 143.43, 161.44. A mixture of **7B** (0.21 g) and phenol (0.93 g) in 33%-HBr AcOH (10 mL) was heated at 75 °C for 20 h and evaporated to dryness under reduced pressure. Dark brown residue was washed several times with ether until colourless and then with ether-methanol mixed solvent to give **8G** as colourless powder (91% yield).

1,18-Diamino-5,14-diazaoctadecane 4HBr (9He). A mixture of **3Ce** (0.15 g) and phenol (0.32 g) in 33%-HBr AcOH (10 mL) was heated at 75 °C for 20 h and evaporated to dryness under reduced pressure. Dark brown residue was washed several times with ether until colourless and then with ether–methanol mixed solvent to give **9He** as colourless powder (94% yield).

1,8-Bis(ethylamino)-N^1,N^8-di(*p*-toluenesulufonyl)octane (4D) and 1,8-bBis(ethylamino)octane 2HBr (5D). A mixture of N^1,N^8-di(*p*-toluenesulufonyl)-1,8-diaminooctane[20] (1.00 g), potassium carbonate (1.54 g) and bromoethane (2.5 mol equiv) in 60 mL of DMF was stirred at rt for 3 days and filtered through Celite. The filtrate was evaporated under reduced pressure and the residue was chromatographed on a silica gel column eluted with chloroform:acetone (98:2 v/v). The fraction indicating $R_f=0.7$ on TLC (chloroform:acetone (98:2 v/v) was collected and evaporated to dryness to afford **4D** (0.90 g, mp 112–113 °C recryst from acetone–methanol, 80% yield). Anal. calcd for $C_{26}H_{40}N_2O_4S_2$: C, 61.38; H, 7.93; N, 5.51. Found: C, 61.35; H, 7.95; N, 5.45. IR(KRS): ν 1350 and 1550 cm^{-1} (SO_2). ^{1}H NMR data: δ 1.10 (t, $J=7.32$ Hz, Et), 3.20 (quar, $J=7.32$ Hz, Et), 1.26 (m, CH_2 of –$(CH_2)_8$–), 1.52 (quin, $J=7.32$ Hz, CH_2 of –$(CH_2)_8$–), 3.10 (t, $J=7.32$ Hz, CH_2 of –$(CH_2)_8$–), 2.42 (s, CH_3), 7.29 (d, $J=8.30$ Hz), 7.68 (d, J = 8.30). ^{13}C NMR data: δ 14.06 (Et), 42.59 (Et), 26.55, 28.67, 29.09, 47.51, 21.45 (CH_3), 127.04, 129.55, 135.91, 137.24, 142.88. A mixture of **4D** (0.88 g) and phenol (20 mol equiv)) in 33%-HBr AcOH (10 mL) was heated at 75 °C for 20 h and evaporated to dryness under reduced pressure. Dark brown residue was washed several times with ether until colourless and then with ether–methanol mixed solvent to give **5D** as colourless powder (96% yield).

1,5-Bis(ethylamino)-N^1,N^5-di(*p*-toluenesulufonyl)pentane (4E) and 1,5-bis(ethylamino)pentane 2HBr (5E). A mixture of N^1,N^5-di(*p*-toluenesulufonyl)-1,5-diaminopentane[20] (1.00 g), potassium carbonate (1.68 g) and bromoethane (0.45 mL) in 60 mL of DMF was stirred at rt for 3 days and filtered through Celite. The filtrate was evaporated under reduced pressure and the residue was chromatographed on a silica gel column eluted with chloroform:acetone (98:2 v/v). The fraction indicating R_f = 0.7 on TLC (chloroform:acetone (98:2 v/v) was collected and evaporated to dryness to afford **4E** (0.90 g, mp 90–91 °C (spontaneous cryst. upon dryness and left standing), 72% yield). Anal. calcd for $C_{23}H_{34}N_2O_4S_2$: C, 59.20; H, 7.34; N, 6.00. Found: C, 59.12; H, 7.38; N, 5.93. IR(KRS): ν 1350 and 1550 cm^{-1} (SO_2). ^1H NMR data: δ 1.09 (t, J = 7.32 Hz, Et), 3.20 (quar, J = 7.32 Hz, Et), 1.32 (m, CH_2 of –$(CH_2)_5$–), 1.57 (quin, J = 7.32 Hz, CH_2 of –$(CH_2)_5$–), 3.10 (t, J = 7.32 Hz, CH_2 of –$(CH_2)_5$–), 2.42 (s, CH_3), 7.29 (d, J = 8.30 Hz), 7.68 (d, J = 8.30 Hz). ^{13}C NMR data: δ 14.01 (Et), 42.76 (Et), 23.67, 28.39, 47.37, 21.47 (CH_3), 127.04, 129.60, 137.09, 142.98. A mixture of **4E** (0.77 g) and phenol (20 mol equiv)) in 33%-HBr AcOH (10 mL) was heated at 75 °C for 20 h and evaporated to dryness under reduced pressure. Dark brown residue was washed several times with ether until colourless and then with ether–methanol mixed solvent to give **5E** as colourless powder (95% yield).

Enzymes and nucleic acids

The wild type T7 RNA polymerase was purchased from GIBCO-BRL. The mutant polymerase F644Y was purified from mutant protein highly expressed *Escherichi coli* clone by Nippon Gene Co., Ltd. The rNTPs and radioactive nucleotide were from Amersham Pharmacia Biotech. The fluorescent 3′-dNTPs were synthesized by Wako Pure Chemical Co., Ltd.

Transcription reaction:[13]

The transcription reaction (total volume 10 μL) was done at 37 °C for 1 h in a mixture (pH 8.0) containing Tris–Cl (40 mM), $MgCl_2$ (8 mM), dithiothreitol (DTT, 5 mM), GMP (200 μM), each of rNTP (250 μM), [α-^{32}P]UTP (0.2 μCi, 3000 Ci/mM), and wild type T7 RNA polymerase (5 units) in the presence of polyamine of interest (2 mM) and the template linear dsDNA (10 μM), which was derived from pBluescript KS(+) plasmid (Stratagene) by PvuII restriction endonuclease. After addition of 10 μL of formamide loading dye, the reaction mixture was denaturated at 90 °C for 2 min, and then subjected to electrophoresis on 8% polyacryl amide gel containing 6 M urea. The radioactivity of RNA products in the dried gel was measured with BAS 2000 image analyzing system (Fuji Photo Film Co., Ltd).

The activation effect by polyamine was assayed by measuring the incorporation rate of the radioactive substrates analyzed with electrophoresis on polyacrylamide gel containing 6 M urea. Electrophoresis for the selected samples is shown in Figure 1. Relative effect of the additive was indicated by 'activity index', which is the quotient of the intensity magnitude of the measured radioactivity in the presence of the additive divided by that of the control transcription assay (in the absence of the additive polyamine). The results are summarized in Table 1.

Transcriptional sequencing

The transcriptional sequencing was performed by the methods reported[12,13] which included recent modification as follows; a sequencing reaction mixture contained PCR product (10 ng) from lambda phage genomic DNA shotgun clone as the sequencing template, the fluorescent 3′-dNTPs, inosine triphosphate (ITP, 2500 μM),[21] UTP (500 μM), ATP (250 μM), CTP (250 μM), PPase (10 units), and T7 RNA polymerases F644Y (25 units).[13] The sequencing reaction was carried out at 37 °C for 1 h. The excess of fluorescent 3′-dNTPs in the labeled product was eliminated by Sephadex G-50 column (Amersham Pharmacia Biotech Co., Inc.). The purified product was analyzed by ABI PRISM 377 XL DNA Sequencer (Perkin Elmer Corp.), as shown in Figure 2.

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