Design, Synthesis, and Anti-HIV Activity of "Multi-Layered" Macromonocyclic Polyamines

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"Multi-layer" cyclic polyamines were designed and synthesized, of which several compounds were found to be potential anti-HIV agents in antiviral activity assays against human T-lymphotropic virus type III (HTLV-IIIb) in infection to MT-4 cells.

Increasing knowledge of the crucial role of natural polyamines, putrescine, spermidine, and spermine, in cell biology has been stimulating versatile basic and applied research interests as reviewed in brain pathology,[1] environmental stress in plant,[2] plant molecular genetic analysis,[3] chemotherapy of parasitic protozoan diseases,[4] cancer chemoprevention,[5,6] ion chanells in the nervous system,[7] and the interaction between PAMAM dendritic polymers and DNA.[8] We designed "multi-layer macrocyclic polyamines" which might be appropriate polymorphismic molecular scaffold as host-molecules in ionic or molecular interaction with small or large guest ions or molecules. Here we would like to describe briefly the result of molecular architecture and the preliminary results of the antiviral activity assays of polycationic compounds against human T-lymphotropic virus type III (HTLV-IIIb).

In our design concept of polymorphismic molecule, several macromonocyclic polyamines with the same and/or different ring size and nitrogen content are connected each other by alkylene spacer with variable length of the chain. Such molecular alignment is expected to be merit in solution for the free rotation of macrocycles around the connecting alkylene chain, which in turn will provide flexible molecular form (*i.e.* polymorphism) optimizable for the best fitting against interacting ions or molecules with various charge and size. Established efficient synthesis was characterized by the elaboration of differential synthesis of the terminal ring from the internal polyamine ring. A key intermediate was *N,N*-bis(3-bromopropyl)benzylamine HBr salt for this purpose, which was useful both for chain elongation and macrocyclization with the tosylamide terminal group (K_2CO_3, r.t. 2 days in DMF) and for providing of juncture amine site(s) to the alkyl chain after catalytic *N*-debenzylation (4 atm-H_2, 70 °C, 10%-Pd/C, 24 h, in AcOH). We have succeeded in preparation of many sets of bis-, tris-, and tetrakis-macromonocyclic polyamines.

Among synthesized single and multi-aligned macromonocyclic polyamines, **1** – **13**,[9] composed of units featured by natural polyamine moieties, were subjected to antiviral activity assays against human T-lymphotropic virus type III (HTLV-IIIb) in infection to MT-4 cells.[10] As standard references, dextran sulfate,[11] 3'-azido-2',3'-dideoxythymidine (AZT),[12,13] 2',3'-dideoxyadenosine (ddA),[12] and 2',3'-dideoxyinosine (ddI)[12] were treated for comparison under the same conditions. The anti-HIV activities are listed in Table 1.

All tested compounds were found to be effective (SI > 1) as anti-HIV agents. It is obvious that nitrogen contents of the compounds appear to be closely dependent on the antiviral activities with respect to EC_{50}; in extreme cases, compound **8** and

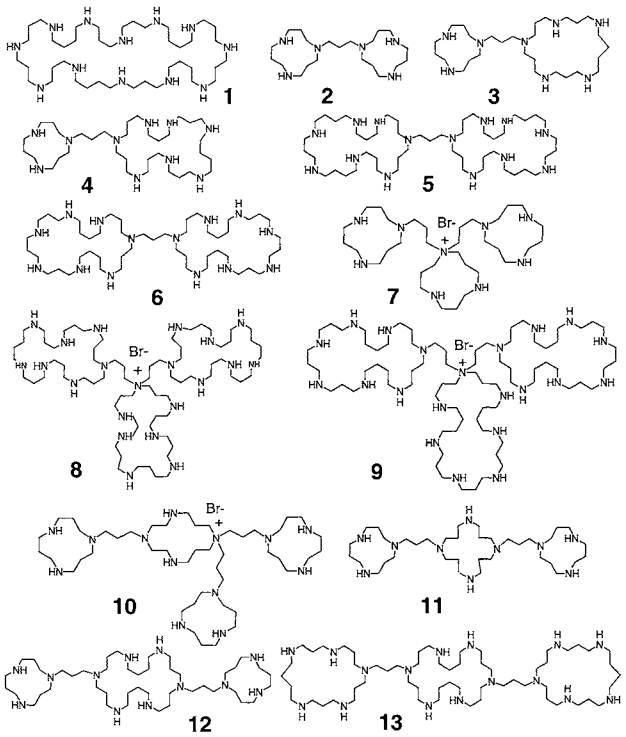

Table 1. Anti-HIV activities of multi-layer cyclic polyamines

Compd	CC_{50} /μM	EC_{50} /μM	SI
1	74.9	3.8	20
2	518	204	3
3	493	27	18
4	127	7.7	16
5	83	1.3	62
6	228	1.2	184
7	470	2.5	191
8	8.6	0.67	13
9	6.9	0.94	7
10	298	9.8	31
11	75.5	1.98	38
12	302	8.4	36
13	47.2	2.3	21
dextran sulfate	>1000	0.84	>1190
AZT	247	0.019	> 13000
ddA	1580	19.2	82
ddI	> 5000	18.6	> 269

CC_{50} ; 50% cytotoxic concentration to the growth of mock-infected MT-4 cells. EC_{50} ; 50% effective concentration against the cytopathicity of HIV-IIIb in MT-4 cells. SI ; selectivity index = CC_{50}/EC_{50}

9 possessing the maximum 21 nitrogen atoms in total were the most potent, and compound **2** with the minimum 6 nitrogen atoms was the least active. Some features emerge in relation to the structural types; in bis-cycles **2** – **5**, the increase in the ring size appears to be in close relation to the decrease in both CC_{50} and EC_{50}. Extraordinary high CC_{50} in **6** compared with that in the positional isomer **5** suggests us that index values would be optimized by thoughtful design of a molecule. In the case of ammonium types **7** – **9**, the increase in the ring size appears to be synchronized with the decrease in CC_{50}. In contrast, ammonium **10** shows high CC_{50} and low EC_{50}. Tris-cycles, **11** – **13**, are moderately effective anti-HIV agents in both indices. In comparison of SI values with those of reference compounds, it is suggested that **6** and **7** are more potent than ddA, and several among the others are moderately potent.

These analyses suggest that the macrocycles in the tris-cycle framework with more nitrogens and proper disposition of spermine-methylene groups appear to be the targets for the antiviral agents of choice valuable for further studies. In comparison with potent anti-HIV activities of bicyclam[14,15] and biscyclam,[16,17] and selective antagonistic binding of bicyclam to the CXC-chemokine receptor CXCR-4, the main coreceptor used by T-tropic virus,[18] the antiviral effect of the present compounds seems to be caused by their polycationic nature similar to that of bicyclam or biscyclam.

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9 Fundamental synthetic methods were based on our previous reports; M. Iwata and H. Kuzuhara, *Bull. Chem. Soc. Jpn.*, **62**, 198 (1989) and **62**, 1102 (1989). Compounds **1** – **13**, pending Japanese patent, were obtained as (water soluble colorless powder) HBr salts by direct deprotection of the corresponding per-*N*-tosylated precursors with a HBr-AcOH-phenol mixture. The total synthetic pathway will be reported in detail elsewhere. Salt structures were unambiguously confirmed by analyses of their IRs and mass spectra employing high resolution mass spectrometry (HRMS) and positive ion fast atom bombardment mass spectrometry (FAB(+)MS). In compound **10**, the position of quarternary amine is still a matter of selection between that shown here and the juncture nitrogen atom on the 12-membered ring.
10 Antiviral activity assays[14,15] were carried out by courtesy of Prof. H. Nakashima (Univ. Kagoshima, when in Department of Microbiology, The Yamanashi Medical University) and Prof. N. Yamamoto (Department of Microbiology and Molecular Virology, The Tokyo Medical and Dental University School of Medicine), and the results were assessed by two factors: 50% cytotoxic concentration (CC_{50}) to the growth of mock-infected MT-4 cells and 50% effective concentration (EC_{50}) against the cytopathicity of HTLV-IIIb in MT-4 cells. Total effectiveness is evaluated by selectivity index ($SI = CC_{50}/EC_{50}$). In definition, compounds with the larger CC_{50} are the less toxic drugs, and those with the less EC_{50} are the more active; thus, compounds with SI higher than one are more or less potential drugs.
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