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Preparation and Evaluation of Novel Sugar Dendritic Gd-DTPA Complexes for MRI Contrast Agents and Phospha Sugars for Anti-tumour Agents

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Abstract. Novel Sugar Dendritic Gd(III)-DTPA complexes for MRI Contrast Agents (CAs) were prepared and evaluated by *in vitro* and *in vivo* methods. The sugar dendritic MRI contrast agents had a good blood vessel pool character and drew blood vessels and liver cancers remarkably clearer and longer time enough than the clinically being used Gd(III)-DTPA complex (Magnevist). Phospha sugar derivatives or phosphorus heterocyclic derivatives provided by functional groups such as epoxide, bromide, etc., were prepared and evaluated by the MTT *in vitro* method. These phospha sugar derivatives showed excellent anti-proliferative effects of leukemia cell lines, e.g., K562 and U937, as well as solid cancer cells in fashions of (i) higher activity, (ii) wider spectra, and (iii) higher selectivity and specificity than Imatinib mesylate (Gleevec), which is one of the most frequently used chemotherapeutical molecular targeting anti-tumour agent.

Introduction

Cancer is the most serious disease. Cardiac and cerebral vascular diseases are the second and the third worst diseases, respectively. Therefore, to diagnose and cure these diseases are medical demands and innovations of medicinal materials for them are important research themes.

To find tumours at the early stage (early diagnosis) highly functionalized MRI contrast agents [1,2] by which very small cancers can be clearly imaged are required. The currently clinically used MRI contrast agent is Gd-DTPA complex (Magnevist) which is safe and the potential one, however, it has poor characters for imaging the blood vessels and cancers. To improve the poor characters of Gd-DTPA to image the cancers as well as the blood vessels (Magnetic Resonance Angiography; MRA), Gd-DTPA was chemically modified by sugars in this paper to recognize components of the blood vessels for wider MRA windows and MR imaging of the cancers for the early diagnosis.

Molecular targeting chemo-therapeutic agents play one of the most important roles in curing the cancers. Imatinib mesilate (Gleevec) is one of the most commonly clinically used chemo-therapeutic agents. The Gleevec has potential activity against the cancers, especially leukemia cells, nevertheless, it has lower activity towards some kinds of the leukemia cells. The Gleevec sometimes faces lower efficiency for the complete cure of larger tumour tissues. Therefore, new researches to develop alternative anti-cancer agents to the Gleevec are steadily demanded.

Phospha sugar is one of the sugar analogues which have a phosphorus atom in place of the ring oxygen atom of normal sugars and is assigned to a category of *pseudo* sugars. They are not yet found in nature, and then all of them reported until now are chemically synthesized. On the other hand, the alternative pseudo sugars, such as *aza*-, *carba*-, and *thia*-sugars [3,4], having a nitrogen, carbon, and sulfur atom, respectively, in the hemiacetal ring of the sugars, are widely known in nature, also chemically synthesized, and modified extensively. Many of them are known to have important biological activities. In this paper we have developed *phospha* sugar chemistry starting from phosphorus heterocyclic compounds, e.g., 2-phospholene derivatives [3,4].

Experimental

Synthesis of 2,3-dibromo-3-methyl-1-phenylphospholane 1-oxide (DBMPP)

CH_2Cl_2 (10 ml) solution of bromine (5.6 eq.) was added dropwise to CH_2Cl_2 (10 ml) solution of 3-methyl-1-phenyl-2-phosholene 1-oxide (1.4 mmol) and Mn(IV) dioxide (2.0 eq.) and the reaction mixture was stirred for 8 h at room temperature. The reaction mixture was worked-up by addition of sodium sulfite aqueous solution, extraction with chloroform, neutralization with $NaHCO_3$ aqueous solution, and removal of the solvent, and then separation of the diastereomers to give 2,3-dibromo-3-methyl-1-phenylphosholane 1-oxides (DBMPPs: 0.37 g) in 75% yield; m.p. (Shimadu Simultaneous DTA-TG Apparatus (DTG-60A50AH)) 189.20 °C; b.p. 280.24 °C; MS (Voyager-DE Porimerix): 349.29 (M - H^+ (Molecular peak - 1); ^{1}H-NMR (JEOL JNM-AL300 (300 MHz) and Hitach R90H (90 MHz); Solvent: $CDCl_3$, δ (ppm)); 1.67 (s, 3H, CH_3), 2.36-2.46 (m, 2H, H-4), 2.97-3.02 (m, 2H, H-5) 4.28-4.31 (m, 1H, C-2), 7.51-7.70 (m, 5H, Ph-H); HPLC (Apparatus: JASCO HPLC Set (JASCO 860-CO, 880-PU, 875-UV, RI-930, and 807-IT; Column: Silica gel

(Analysis: Wakopac, Wakosil Φ 4.6 mm × 250 mm, Eluent: $CHCl_3$: MeOH = 30 : 1, Flow rate: 0.5 ml/min), RT (retention time: min) values of diastereomers were 8.1, 9.1, 9.9, and 11.5 (Fig. 4).

Results and Discussions

The molecular size of the MRI contrast agent of Gd-DTPA complex (Gadolinium Diethylenetriamine pentaacetic acid: Magnevist) (Fig. 1) is small and then the contrast agent penetrates the blood vessels. To make the MRI contrast agent remain in the blood vessels so as to image the clearer blood vessels (Magnetic Resonance Angiography (MRA)) and tumours, the sugar ball dendritic structure of the Gd-DTPA complexes were designated and synthesized (Scheme 1 exemplifies Gd-DTPA-D1-Glc(OAc) (four peracetylated glucose derivative)) [1,2]. The product of the alkaline hydrolyzed product of the acetate derivative, Gd-DTPA-D1-Glc(OH), is represented here as DEN-OH.

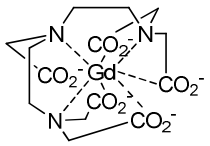
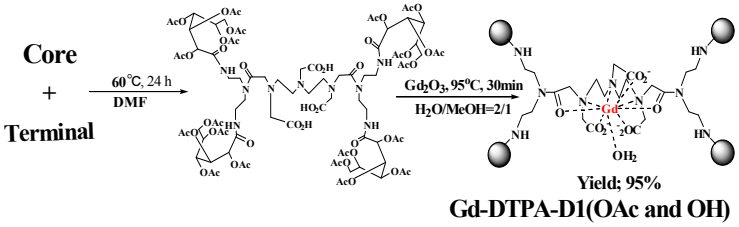


Figure 1. Gd-DTPA (Magnevist).



Scheme 1. Preparation of Gd-DTPA-D1-Glc (OAc) and DEN-OH.

The *in vivo* evaluations of DEN-OH compared with Gd-DTPA for MRA and liver cancers are shown in Fig. 2 and 3.

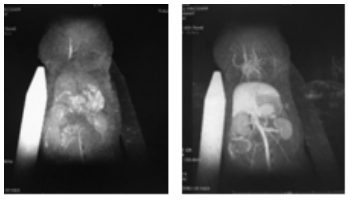


Figure 2. MRA of rats at 30 min after injection (Left: by Gd-DTPA; Right: by DEN-OH).

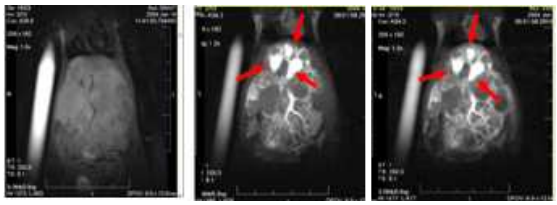
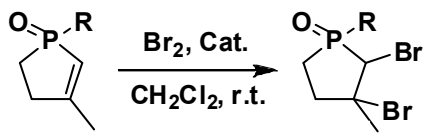


Figure 3. MRI of the liver cancers of rats (Left: by Gd-DTPA at 3 min after injection; Right two: by DEN-OH at 3 and 30 min after injection).

Addition of bromine to the unsaturated C=C double bond of the starting 2-phospholene derivatives produced 2-bromo- or 2,3-dibromophospholane derivatives. The substitution reaction of 2-bromophospholane derivatives, which correspond to 2-bromo-2-deoxyphospha sugar derivatives, with amine nucleophiles gave *N*-glycosides of *phospha* sugar derivatives. Further, nucleic acid bases such as uracil were introduced into 2-phospholene 1-oxide derivatives by the cyclization reaction of acrylamide derivatives to prepare *phospha* sugar nucleosides [3,4].

The biological activities for these *phospha* sugars or phosphorus heterocycles by *in vitro* MTT evaluations were carried out and the *in vivo* bio-assay is planned. The preparation and the *in vitro* MTT evaluation of the dibromodideoxyphospha sugar derivative (DBMPP) against U937 cell lines are shown in Scheme 2 and Fig. 4. The phospha sugar derivative showed the excellent results compared with Gleevec [5] based on the *in vitro* evaluation data. The phospha sugar exert higher

activity than Gleevec against U937 cells and the derivative did not give any damage against normal leukocyte.



Scheme 2. Preparation of 1,2-dibromo-1,2-didoxyphospha sugars (DBMPP).

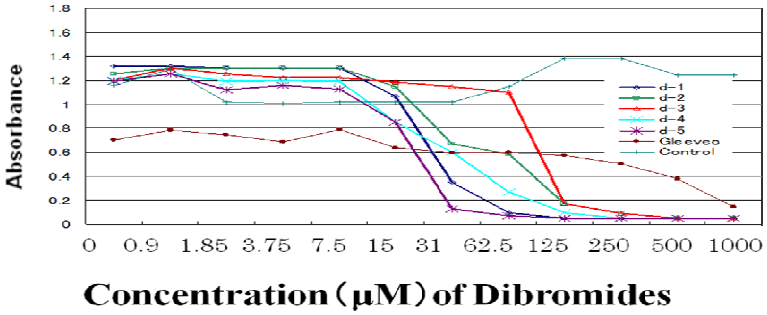


Figure 4. MTT evaluation of *phospha* sugars, (DBMPPs: four diastereomers and mixture: from d-1 to d-5) as anti-tumour agents (comparison with Gleevec) against U937 cells.

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