

## Biomolecular approach to the design of potential drugs\*

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**Abstract:** The approach to drug design on the basis of molecular-level information on biological processes is being driven by the expanding knowledge of the details of molecular events in biological systems. We have directed attention to the design of potentially active compounds based on the aforementioned “biomolecular” concepts. Selected examples from our studies are discussed.

This paper presents three case studies of approaches to the development of potential medicinal agents whose design has evolved from considerations of *molecular mechanisms* of processes in selected biological systems.

**Keywords:** biomolecular chemistry; DNA biosynthesis; enzyme inhibitors; imino sugars; glucocerebrosidase.

### NUCLEIC ACID MIMICS

The unraveling of the structure of deoxynucleic acid (DNA) [1] ushered in a new area in which a fundamental link was established between molecular chemistry and biology. The double-stranded structure of DNA, in which the nucleic acid chains are held together via hydrogen bonds between specific nucleobases, constitutes the foundation of our current understanding of the molecular mechanisms of genetic processes. This knowledge has been the fountain of spectacular developments at the frontiers of chemistry and biology during the past half-century.

The imperative role of specific hydrogen bonding, between the nucleobases, in the replication, transcription, and translation processes associated with genetics, poses the following intriguing question. Can polymers composed of nucleoside analogs in which natural nucleobases are attached to molecular units other than the sugar-phosphate moiety (Fig. 1) exhibit biologically relevant Watson–Crick interactions with natural nucleic acids? This question was addressed in a program on the study of *nucleic acid mimics* undertaken in our research group. The initial phase of the program visualized two types of monomeric building blocks for the construction of polymeric chains bearing nucleobases in a desired sequence (Fig. 2). Monomers of type **1** consisted of units based upon the assembly of nucleobases as branches upon natural amino acids [2], it being projected that linking of the resulting amino acid functionalities—via amide bonds—would result in nucleic acid mimics in which potentially a large degree of structural variety could be introduced. In systems of type **2**—where the bases are attached to diols—the possibility of chain formation was visualized via direct condensation of the hydroxyl functions or through the intermediacy of appropriate spacers.

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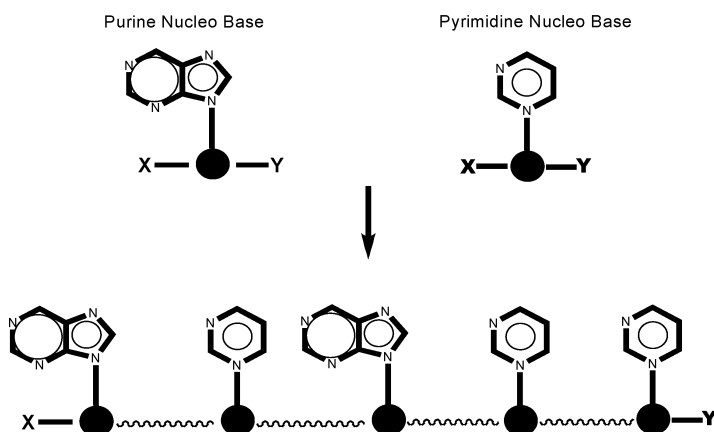


Fig. 1 Nucleic acid mimics.



Fig. 2 Building blocks of nucleic acid mimics.

Several representatives of building blocks corresponding to **1** (Fig. 3) and a few of the corresponding peptides were synthesized and their physical and biological properties examined [2]. However, this early work, which in retrospect was ahead of its time, remained limited in its scope and implications. Later developments in this field have opened up the subject of polyamide nucleic acids (PNAs), which is fast emerging as an important area of biological research [3,4]. The use of PNAs as diagnostics is already established, and their application in the field of medicine is anticipated [3]. Monomers corresponding to structural type **2** are exemplified by nucleoside analogs **3a–e** (Fig. 4) [5]. The 1,3-dihydroxy system in these monomers mimicked the relative location of the hydroxyl groups in deoxyribose, while the chain itself can be folded to resemble in part the shape of the sugar moiety. Compounds **3a–e** were synthesized in the context of this program and constitute examples of some of the earliest reported acyclic nucleosides [5]. Subsequent biological studies of these analogs have led to the development of the guanosine derivative **3e** as the antiviral agent *peniclovir* and its biological precursor *famciclovir* [6]. The activity of *peniclovir* is attributed to its phosphorylation (triphosphate) and subsequent incorporation in nucleic acids. It should be noted that these molecules are structurally C-nucleoside analogs and are, as expected, more stable than their counterparts with an aminal linkage (e.g., Aciclovir).

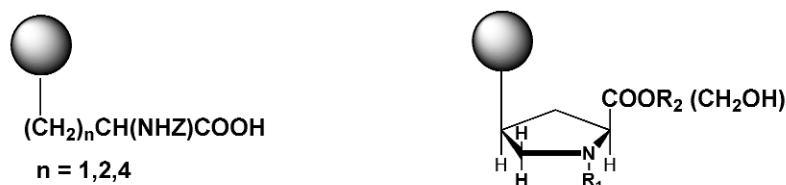


Fig. 3 Polyamide nucleic acid building blocks (PNA monomers).

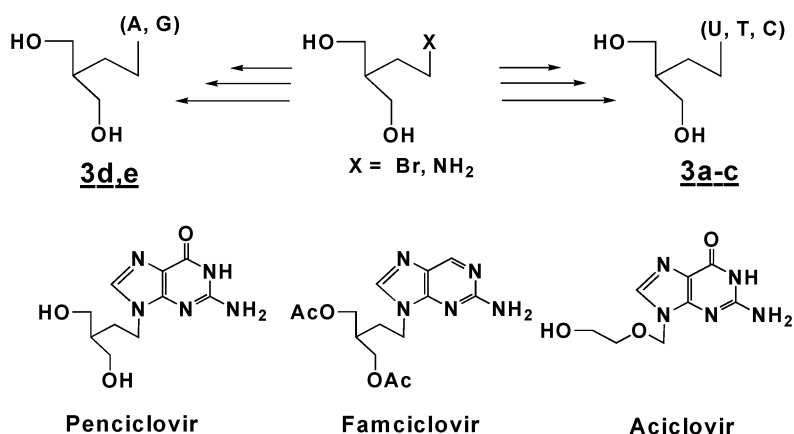


Fig. 4 Acyclic nucleoside analogs.

### THYMIDYLATE SYNTHASE INHIBITORS

The molecular mechanisms of the biosyntheses of nucleic acids constitute a potentially fruitful field for drug design based on the development of directed enzyme inhibitors. In the field of cancer, the clinically utilized drugs 5-flourouracil and methotrexate exercise their anticancer mode of action by interfering with the enzymes involved in the thymidylate synthase (TS) cycle—a sequence of molecular transformations—that converts deoxyuridine phosphate to deoxythymidine phosphate, an essential building block of DNA (Fig. 5) [7]. The methyl group for this transformation is derived from the folate cofactor methylenetetrahydrofolate. The delivery of a “methyl” group by the cofactor—which itself is devoid of a methyl moiety—is an unprecedented chemical reaction. The mechanism of the methyl transfer has been extensively studied [8] and established as shown in Fig. 6. As the mechanism shows, the methylene unit is derived from the imidazolidine ring of the cofactor, whereas the tetrahydropteridine moiety (of the cofactor) provides a hydride equivalent to the crucial exocyclicmethylene intermediate for completion of the process. That this chemistry is not uniquely a prerogative of a biolog-

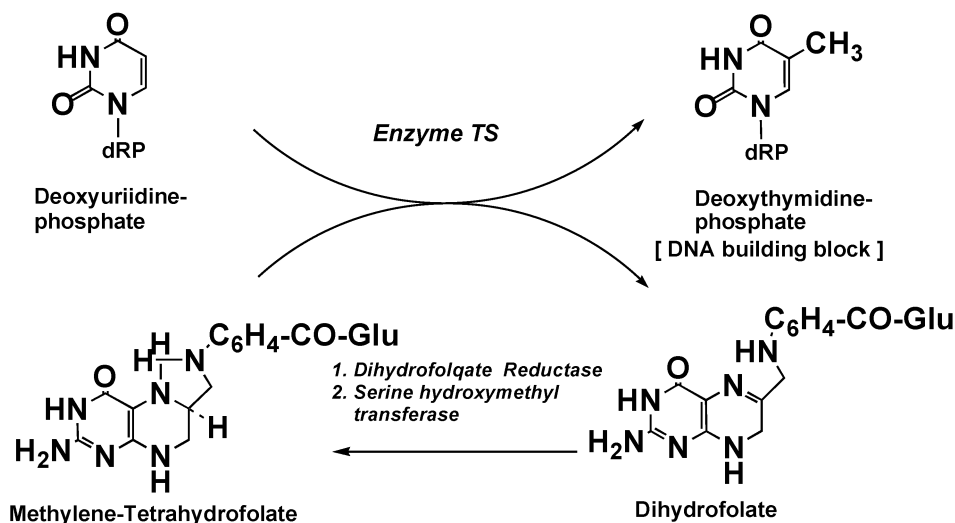


Fig. 5 Thymidylate synthase (TS) cycle.

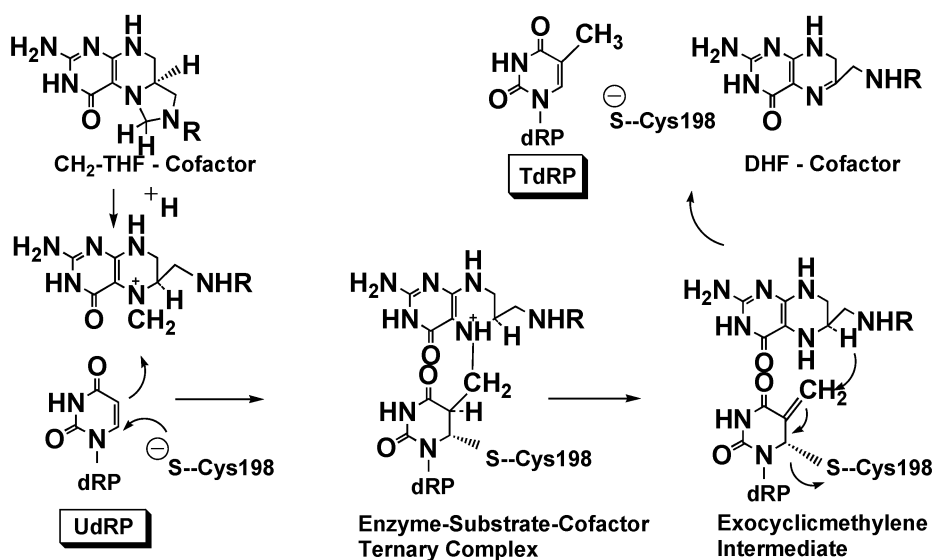


Fig. 6 Mechanism of the TS reaction.

ical process was shown in our laboratory. A cofactor “analog” containing an imidazolidine moiety fused to a dihydrophenanthridine unit was designed, and it was demonstrated that this analog can transfer a *methyl unit* to the 5-position of an activated uracil derivative [9]. The mechanism of the model reaction closely followed that of the enzymatic process and represents the first case of an overall non-enzymatic methyl group transfer from an imidazolidine derivative. It may be added in passing that the methylene transfer step can be mimicked in a variety of suitably substituted imidazolidines and has been developed in our laboratory into a synthetic strategy for the practical transfer of -CHR- units to mono- and bi-nucleophiles [10].

The elucidation of the molecular mechanism of the TS reaction suggests new approaches for designing inhibitors of a specific step of the TS cycle. It was projected that a uracil derivative bearing a thioether (SR) function at the C-6 position could enter the TS cycle and develop into the corresponding “exocyclicmethylene” intermediate (Fig. 7) carrying a *thioacetal* function at C-6. In the subsequent reduction step, the hydride transfer could—in the case where SR is a good departing group—eliminate an

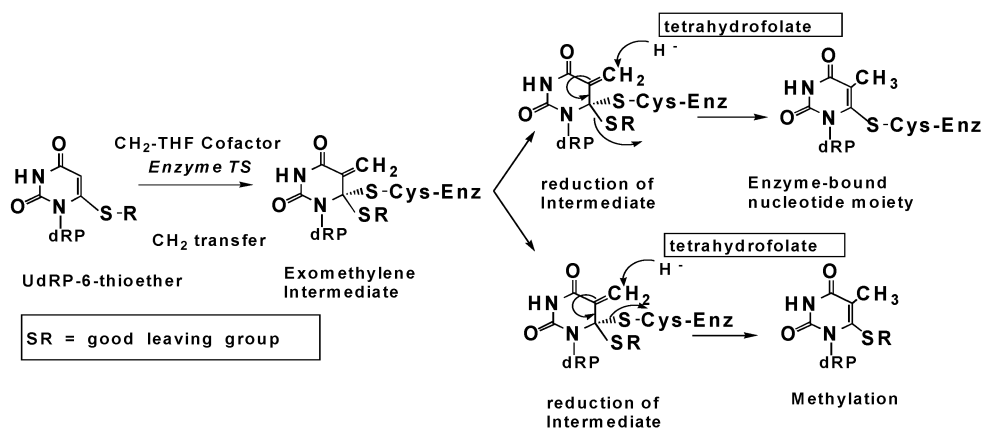


Fig. 7 Design of enzyme-bound TS inhibitor.

SR anion in a process concomitant with the hydride transfer step. The latter process should lead to a covalent binding of the pyrimidine moiety to the apoenzyme, via the Cys-189 residue. This reaction sequence would result in inhibition of the TS enzyme. Model studies have shown that the analogous thiol exchange can be duplicated in non-enzymic reactions [11]. Based on these results, pyrimidine derivatives **A–E** (Fig. 8) have been synthesized and subjected to biological evaluation [11]. The preliminary results shown in Fig. 8 are of potential interest. However, it should be pointed out that at present the mechanism of the observed activities is not known.

	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>
L 1210	73.8 ± 22.3	43.8 ± 22.3	> 500	> 500	8.20 ± 0.52
Molt 4/8*	27.6 ± 11.0	41.4 ± 6.2	> 500	> 500	8.67 ± 0.12
CEM/O*	41.4 ± 6.2	27.6 ± 11.0	> 500	> 500	7.27 ± 0.12

\*Human T-lymphoblast cell

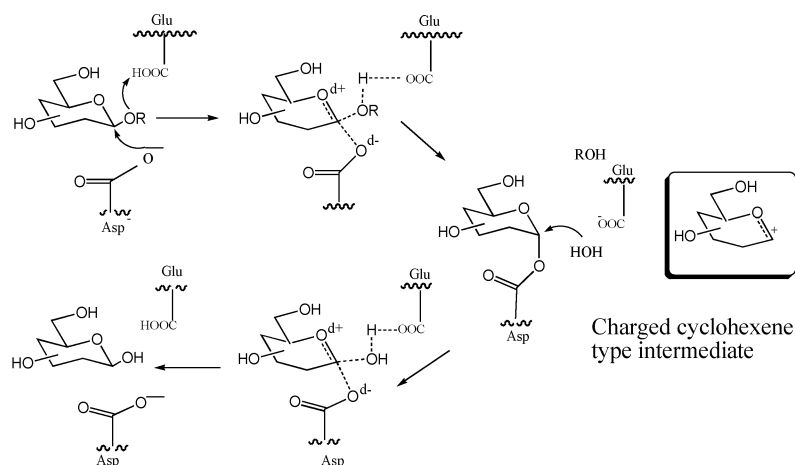
R = SiDMTB

**Fig. 8** Activity of nucleoside analogs\* (IC<sub>50</sub> μM). Courtesy of Prof. E. de Clerg, Rega Institute of Medical Research, Leuven, Belgium.

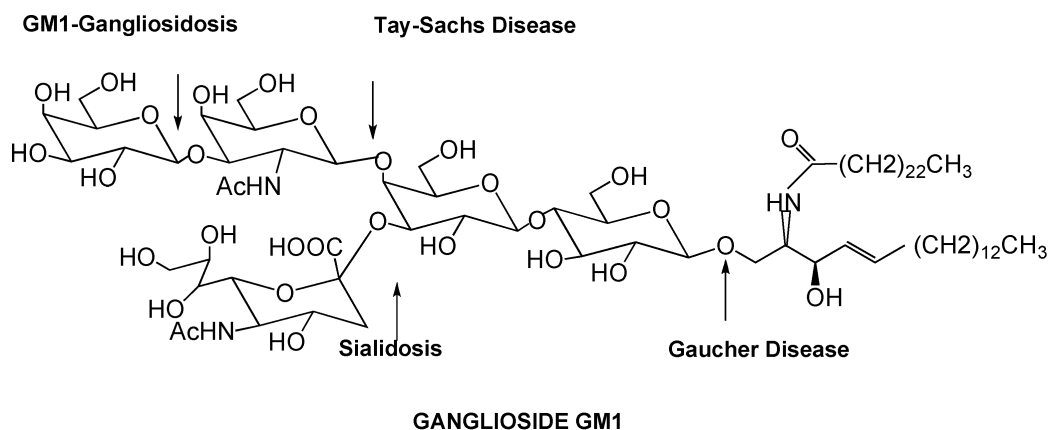
## GLYCOSIDASE INHIBITORS

The concept that transition-state analogs (TSAs) of enzyme-catalyzed transformations bind preferentially to the active site of the enzymes has found widespread application in designing enzyme inhibitors [12]. The TSA concept is also fundamental to the development of catalytic antibodies [13]. Glycosidases represent a class of enzymes whose mechanism of action is associated with the involvement of a crucial transition state which resembles a flattened (pyranyl) six-membered ring with a partial positive charge on the oxygen (Fig. 9). This, amongst other lines of evidence, is attested by the inhibition of many glycosidases by five- and six-membered imino sugars [14]. In the latter compounds, the nitrogen in the protonated state serves as the source of the positively charged site on the ring. It is noteworthy in this context that *N*-hydroxymethyldeoxynojirimycin (Diastabol<sup>®</sup>) and *N*-butyldeoxynojirimycin (OGT-918) are currently marketed as drugs. The former for diabetes and the latter, under the trade name Zavesca, are currently sold in the United States, Canada, and much of the European Union (as of May 2005) for treatment of type I Gaucher's disease.

The deficiency of specific glycosidases involved in the hydrolysis of glycosphingolipids is responsible for several inherited disorders [15]. In Fig. 10, the genetic diseases associated with the various sites (arrows) of hydrolysis of glycosidic linkages in ganglioside GM<sub>1</sub> are indicated. Gaucher's disease, which involves the deficiency of glucocerebrosidase (Fig. 10), results in the accumulation of the substrate glycoside leading to the consequential clinical manifestations. The condition can be potentially alleviated either by suppressing the biosynthesis of the glucocerebrosidase or by enhancing the availability of the glucocerebrosidase enzyme. Currently, the disease is mainly treated by enzyme replacement therapy, using a human ceredase. In addition, as mentioned earlier, *N*-butyldeoxynojirimycin (Zavesca), which suppresses the synthesis of glucocerebrosidase from its biological building blocks (inhibition of glucoceramide synthase) has been clinically applied. As can be expected, the therapy using



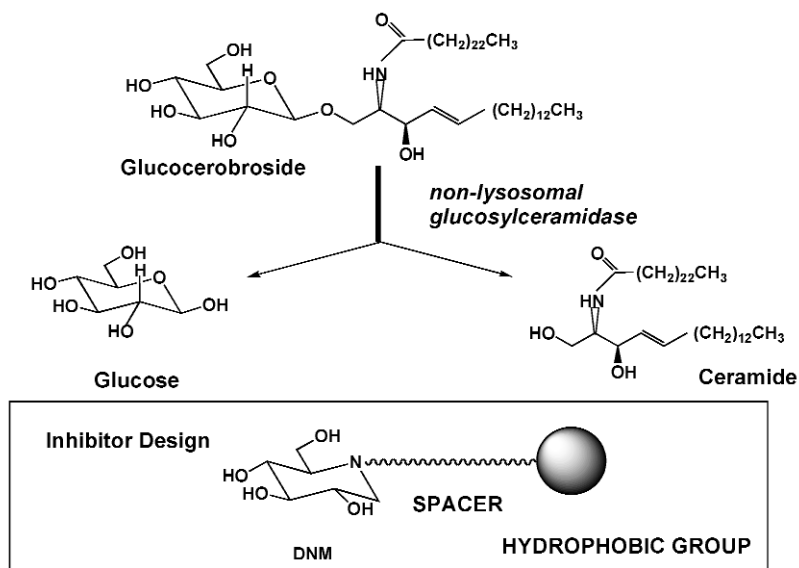
**Fig. 9** Mechanism of glycosidases.



**Fig. 10** Hydrolysis of glycosphingolipids.

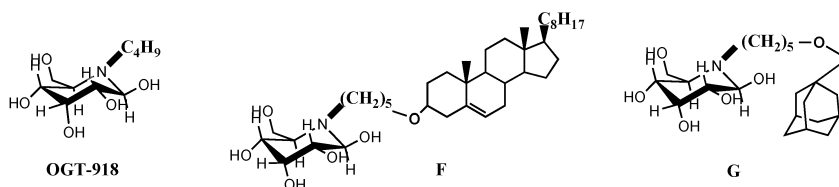
the human enzyme is extremely costly, a factor which has placed Gaucher's disease in the category of the so-called "orphan diseases". Investigation of the topic of Gaucher's disease at the Medical Faculty of the University of Amsterdam has led to the discovery of a membrane-bound non-lysosomal glucosylceramidase [16] (Fig. 11). This enzyme is located near the cell surface and exhibits a different specificity from lysosomal glucocerebrosidase. It was of interest to study the inhibition of the non-lysosomal enzyme with the aim of examining its direct or indirect role in the sphingolipid metabolism linked to ceramide-mediated signaling processes. In order to develop a potential specific inhibitor, consideration was given to designing deoxynojirimycin derivatives in which a large hydrophobic group was attached to the deoxynojirimycin nitrogen via a suitable spacer (Fig. 11).

It was rationalized that while the deoxynojirimycin unit could be potentially responsible for the inhibitory function, the hydrophobic end would be expected to enhance the affinity of the inhibitor to the membrane binding the enzyme. Based on this projection, a series of molecules corresponding to the inhibitor design visualized in Fig. 11 were synthesized and their inhibitory properties examined [17]. It was found that such derivatives did indeed specifically inhibit non-lysosomal glucosylceramidase and that the inhibitory activity was dependent upon the length of the spacer, its polar character, and, importantly, on the structure of the hydrophobic moiety.



**Fig. 11** Design of non-lysosomal glucosylceramidase inhibitor.

Of the deoxynojirimycin derivatives examined, the compounds **F** and **G** (Fig. 12) were found to exhibit significant activity—the adamantane derivative **G** being the most active inhibitor in the series. The relevant data for the compounds **F** and **G** and for *N*-butyldeoxynojirimycin (OGT-918) are presented in Fig. 12. The inhibitory data indicates that the symmetrical and compact adamantane moiety is presumably ideally suited for binding to the membrane when it is separated from the deoxynojirimycin nitrogen by an oxapentamethylene bridge. It is noteworthy that as an inhibitor for the non-lysosomal glucosylceramidase, compound **G** is about 100 times more active than OGT-918. For the



DNM derivative	<i>Ceridase</i>	<i>lysosomal glucoceribrosidase</i>	<i>non-lysosomal glucosylcerimidase</i>	<i>lysosomal α-glycosidase</i>
OGT-918	912	424.4	0.31	6.43
<b>F</b>	0.96	0.77	0.097	7.20
<b>G</b>	0.16	0.048	0.0017	0.87
<b>Km</b>	3.25	1.45	3.28	1.88

$IC_{50}$  constants are expressed in micromolar. The  $K_m$  values for specific enzymes for 4-methylumbelliferyl- $\beta$ -glucoside for  $\beta$ -glucosidases and 4-methylumbelliferyl- $\alpha$ -glucoside for  $\alpha$ -glucosidase are expressed in millimolar.

**Fig. 12** Inhibition of glycosidases by deoxynojirimycin derivatives.

same enzyme in cultured melanoma cells, the  $IC_{50}$  values are 0.3 nM and 200 nM for **G** and OGT-918, respectively. Furthermore, **G** was 500 times more effective than OGT-918 as an inhibitor of non-lysosomal glucosylceramidase in macrophages. Studies also indicate that at concentrations where **G** completely inhibits non-lysosomal glucosylceramidase, the activity of glucosylceramide synthase is unaffected. Directed experiments indicate that inhibitor **G** is strongly bound and is not easily removed from the enzyme or its surrounding membrane. The investigation of related deoxynojirimycin derivatives in the context of Gaucher's disease are currently in progress at the Medical Faculty of the University of Amsterdam and at the University of Leiden.

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