Chapter 9

Summary

Despite the advances in human knowledge, we still encounter new and more dangerous infectious or oncogenic diseases, which can not be treated using presently available drugs. Another problem lies in the ability of microorganisms and tumors to acquire resistency to pharmacological treatment. The development of new and more potent biologically active substances is, therefore, a crucial task.

This fact also lies behind this thesis. This work is extending the synthesis of acyclic nucleoside phosphonates, from which many are already used in clinical medicine(Hepsera, Viread, Vistide). We concentrated on so called acyclic nucleoside bisphosphonates (ANbPs), a group of relatively sparsely explored compounds. The main aim of this dissertation was to prepare new ANbPs and to assess their biological activity.

During the course of this work we prepared several different series of bisphosphonates. Tests for biological activity were carried out for many of them. In some cases, the evaluation of the activity is still in progress. The main synthetic strategy used was alkylation of nucleobases with a bisphosphonate building block prepared in advance. Further processing of the product by means of cleavage of ester protecting groups lead to free bisphosphonic acids or their sodium salts. These compounds were then subjected to biological screening.

First we synthesised symmetric ANbPs based on glycerol which could be also considered as analogues of nucleotide bisphosphate antagonists of the $P2Y_1$ receptor (Chapters 3 and 4).[1,2] These substances were found to be inactive against DNA, RNA, and herpesviruses. They do not possess any cytostatic activity and so far we have not found any evidence of their action as possible antagonists of the $P2Y_1$ receptor.

Due to their very high polarity, the penetration of ANPs and ANbPs into the cell is severely hindered. We therefore tried to prepare their lipophilic derivatives to make their membrane transport easier (Chapter 5). The lipophilic chains were attached to already known compounds containing PME chain (adenine, guanine, cytosine), and to glycerol based ANbPs (adenine). Antiviral and cytostatic screening revealed significant increase in the activity of the lipophilic derivatives, when compared to free acids.[3] As a surprise it was also found, that lipophilic drivatives attached to adenine are active against Coxsackie virus B4 (RNA virus). In general, in this series of ANPs the activity against RNA viruses is very rare.

In the following project, described in detail in Chapter 6, we studied ANbPs with chiral chain. We prepared two basic enantiomeric series of free phosphonic acids. The mechanism of the alkylation reaction was verified and the absolute configuration of the products was assigned. As in previous cases, one of the main goals was to assess the potential biological activity of these compounds. The additional goal was to study the effect of introduction of chiral centers to synthesized compounds, since the chirality seems to play an important role in EI-complex formation in certain enzymes. Neither antiviral nor cytostatic activity was found. This fact was not particularly surprising since the presence of four negative charges due to phosphonate groups indicated possible problems in membrane transport. To overcome this hindrance, we used liposomes, which are generally used for the transport of highly polar substances inside the cells. Initial cytostatic screening shows very promising activity for the *(S,S)* guanine derivative SV327 (compound **29b**, Chapter 6. The *(R,R)* derivative **29a**, on the other hand, does not exhibit any activity. Further testing is currently in progress.[4]

The last (but not least) part of our investigation of ANbPs concerned the synthesis of geminal (methylene) bisphosphonates (Chapter 7). Substances from this group (*e.g.*, alendronate, ibandronate, zoledronate) have received much attention due to their use in the treatment of osteoporosis and other bone diseases. It was our aim to study biological activity (not limited to osteoporosis) of similar compounds. We, therefore, prepared series of new ANbPs with alendronate side chain. We also synthesized acyclic nucleoside phosphate-phosphonates, emerging from the molecular rearrangement of the original bisphosphonate. We were also able to isolate α-iodophosphonates, as byproducts of the final removal of protecting ester groups from phosphate-phosphonate derivatives. Testing of these substances as potential agents for osteoporosis treatment, as well as antiviral and cytostatic tests are being carried out.