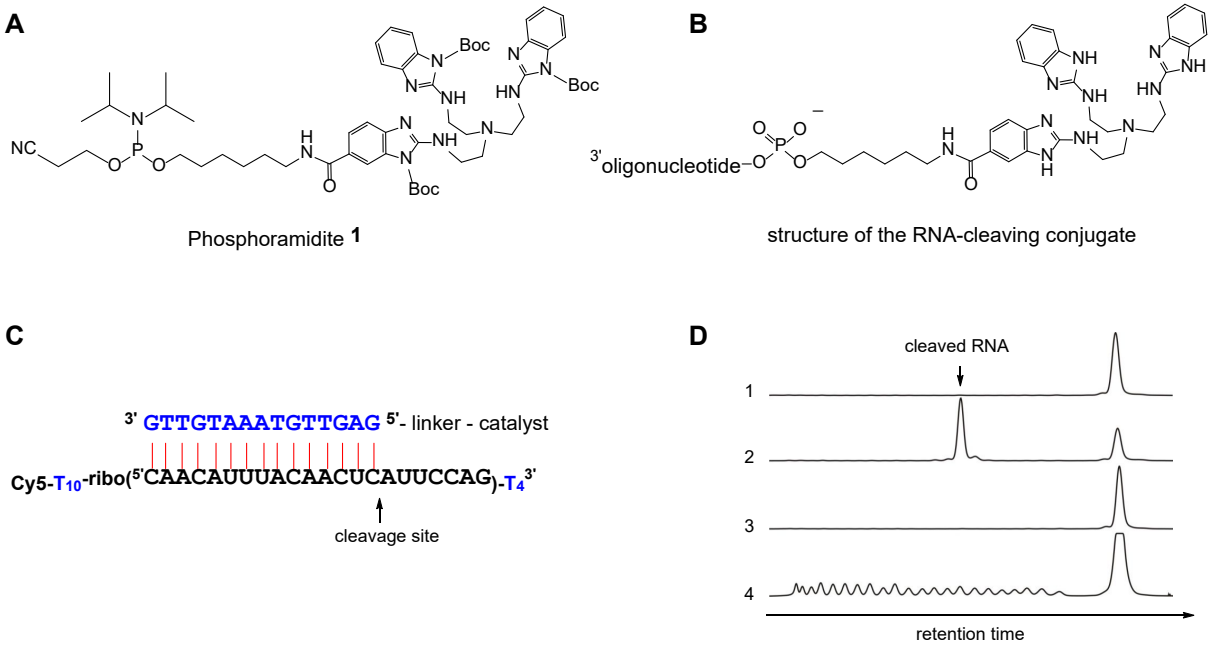
Chemical Biology of Nucleic Acids: Synthesis of Functional Molecules Acting on DNA and RNA.

Development of site-specific artificial ribonucleases



Many years ago, we investigated anion receptor molecules composed of guanidinium ions. Such compounds formed host-guest complexes and accelerated phosphoryl transfer reactions when conducted in DMF to promote ion pair formation. However, RNA cleavage under physiological conditions was hardly catalyzed by simple guanidines. It was found later that heterocyclic guanidine analogs with pK_a values shifted towards neutrality (e.g. 2-aminobenzimidazoles) are much better suited for this purpose. This observation finally led us to the development of phosphoramidite **1** (**A**). Compound **1** can be attached in high yield to the 5' end of DNA or LNA oligonucleotides (**B**). Panel **C** depicts a duplex formed by such DNA-catalyst conjugates and dye-labeled RNA substrates (deoxy-nucleotides in blue and ribonucleotides in black letters). **D** shows the resulting cleavage experiment, analyzed by gel electrophoresis on an ALFexpress sequencer (Lane 1: RNA substrate; lane 2: RNA substrate after incubation with the complementary DNA-catalyst conjugate; lane 3: RNA substrate after incubation with a non-complementary DNA-catalyst conjugate; lane 4: hydrolysis ladder of the RNA substrate. Comparison with lane 2 identifies the cleavage site of the conjugate). Replacing DNA nucleotides by LNA in selected positions further enhances RNA cleavage (substrate half-life about 3.5 h). Such conjugates can be used as universal artificial RNA scissors without sequence restrictions for all kinds of analytical purposes.

Selected references:

1. Danneberg, F., Westemeier, H., Horx, P., Zellmann, F., Dörr, K., Kalden, E., Zeiger, M., Akpinar, A., Berger, R., and Göbel, M. W. (2021). RNA Hydrolysis by Heterocyclic Amidines and Guanidines: Parameters Affecting Reactivity. Eur. J. Org. Chem., 6358–6366. https://doi.org/10.1002/ejoc.202100950

2. Zellmann, F., and Göbel, M. W. (2020). A trisbenzimidazole phosphoramidite building block enables high-yielding syntheses of RNA-cleaving oligonucleotide conjugates. Molecules *25*, 1842. https://doi.org/10.3390/molecules25081842

3. Zellmann, F., Thomas, L., Scheffer, U., Hartmann, R. K., and Göbel, M. W. (2019). Site-Specific Cleavage of RNAs Derived from the PIM1 3'-UTR by a Metal-Free Artificial Ribonuclease. Molecules *24*, 807. https://doi.org/10.3390/molecules24040807

4. Dogandzhiyski, P., Ghidini, A., Danneberg, F., Strömberg, R., and Göbel, M. W. (2015). Studies on tris(2-aminobenzimidazole)-PNA based artificial nucleases: a comparison of two analytical techniques. Bioconjugate Chem. *26*, 2514–2519. https://doi.org/10.1021/acs.bioconjchem.5b00534

5. Danneberg, F., Ghidini, A., Dogandzhiyski, P., Kalden, E., Strömberg, R., and Göbel, M. W. (2015). Sequence-specific RNA cleavage by PNA conjugates of the metal-free artificial ribonuclease tris(2-aminobenzimidazole). Beilstein J. Org. Chem. *11*, 493–498. https://doi.org/10.3762/bjoc.11.55

6. Gnaccarini, C., Peter, S., Scheffer, U., Vonhoff, S., Klussmann, S., and Göbel, M. W. (2006). Site-Specific Cleavage of RNA by a Metal-Free Artificial Nuclease Attached to Antisense Oligonucleotides. J. Am. Chem. Soc. *128*, 8063–8067. https://doi.org/10.1021/ja061036f

7. Scheffer, U., Strick, A., Ludwig, V., Peter, S., Kalden, E., and Göbel, M. W. (2005). Metal-free catalysts for the hydrolysis of RNA derived from guanidines, 2-aminopyridines, and 2-aminobenzimidazoles. J. Am. Chem. Soc. *127*, 2211–2217. https://doi.org/10.1021/ja0443934

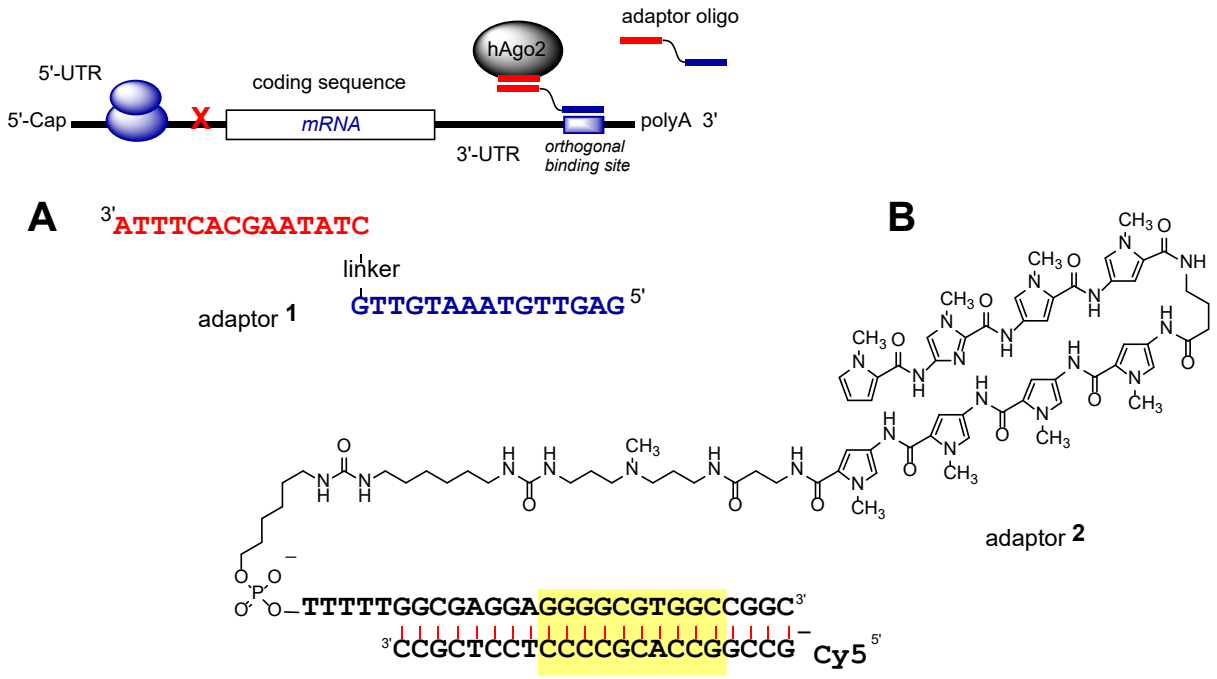
8. Pitsch, S., Scheffer, U., Hey, M., Strick, A., and Göbel, M. W. (2003). On Facts and Artefacts: The Difficulty to Evaluate an Artificial Nuclease. Helv. Chim. Acta *86*, 3740–3752. https://doi.org/10.1002/hlca.200390316

9. Kurz, K., and Göbel, M. W. (1996). Hydrolytical cleavage of TAR-RNA, the trans-activation responsive region of HIV-1, by a bis(guanidinium) catalyst attached to arginine. Helv. Chim. Acta *79*, 1967–1979. https://doi.org/10.1002/hlca.19960790719

10. Gross, R., Bats, J. W., and Göbel, M. W. (1994). trans-Decalin as a spacer for axially configurated diamines: preparation of a phosphate binding bis(guanidinium) compound. Liebigs Ann. Chem., 205–210. https://doi.org/10.1002/jlac.199419940215

11. Gross, R., Dürner, G., and Göbel, M. W. (1994). Acceleration of substitution reactions of a phosphoric acid diester by bis(guanidinium) compounds. Liebigs Ann. Chem., 49–58. https://doi.org/10.1002/jlac.199419940110

Redirection of RNA/DNA-binding proteins by synthetic adaptor molecules



A. Micro RNAs (miRs) are embedded in Argonaute proteins to form RISC complexes (RNA induced silencing complex). Binding of such RISCs to the 3'-UTR of mRNAs results in a downregulation of protein production, in part by blocking translation, in part by induced degradation of the bound mRNA. This mechanism is responsible for the fine-tuning of gene expression and affects a large number of human genes. A RISC complex charged with a specific miRNA (symbolized here by red color) would bind to the complementary "red" RNA sequence, not present in the mRNA example shown above. This mRNA, however, has a different binding site, symbolized by blue color. Adaptor **1** consists of a first oligonucleotide part which matches the RISC complex ("red"). The second part is complementary to the "blue" sequence of the mRNA. Thus, adaptor **1** mediates indirect binding to the 3'-UTR: the "red" RISC is redirected to the non-native "blue" binding site. Specifically, the 3' end of adaptor **1** ("red") binds to miR-20a while the 5' end targets a sterically accessible site in the mRNA of the proto-oncogenic kinase PIM1 ("blue"; DNA nucleotides are represented by regular, LNA nucleotides by underlined letters).

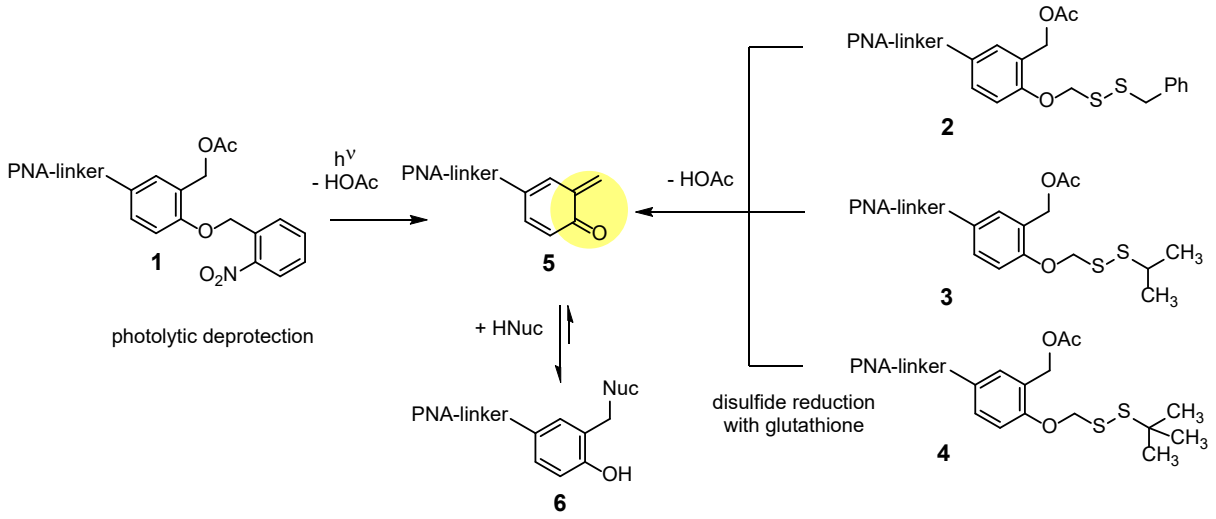
B. The human transcription factor SP1 binds to a GC rich consensus sequence. Adaptor molecule **2** was synthesized to redirect this protein to a non-cognate site rich in AT. The adaptor consists of a DNA duplex (SP1 consensus sequence highlighted in yellow) and a DNA-binding hairpin polyamide. The problem of self-complexation was solved by eliminating all AT rich sequence elements from the DNA part of **2**: the hairpin polyamide selectively binds to the minor groove of TTGTTA and related duplexes. Adaptor mediated recruitment of SP1 was demonstrated by pulldown experiments with a biotinylated capture DNA containing TTGTTA and subsequent western blots.

Selected references:

1. Bolz, M., Scheffer, U., Kalden, E., and Göbel, M. W. (2021). Redirection of the Transcription Factor SP1 to AT Rich Binding Sites by a Synthetic Adaptor Molecule. Helv. Chim. Acta. *104*, e2100095. https://doi.org/10.1002/hlca.202100095

2. Bolz, M., Thomas, L., Scheffer, U., Kalden, E., Hartmann, R. K., and Göbel, M. W. (2020). Redirection of miRNA-Argonaute Complexes to Specific Target Sites by Synthetic Adaptor Molecules. Chem. Biodiversity *17*, e2000272. https://doi.org/10.1002/cbdv.202000272

Site-directed crosslinking of RNA by quinone methides conjugated to PNA



Crosslinking of RNA to complementary oligonucleotides is the chemical background of important bioanalytical protocols and may also help to recruit and redirect Argonaute proteins charged with specific micro RNAs (see above). For the covalent capture of RNA, we have used conjugates of the general structure **5**: the PNA part (peptide nucleic acids) ensures sequence specific hybridization with the target and the quinone methide (highlighted in yellow) as a potent electrophile reversibly alkylates nucleophilic sites within the RNA (RNA nucleotides shown in black, PNA nucleotides in magenta). Quinone methides, however, are labile intermediates that cannot be kept in stock. They must be formed from appropriate precursors directly before use (QMP: quinone methide precursor). In a first approach, we have investigated the conjugate **1**. This compound is completely stable in the dark. Upon irradiation, however, the nitrobenzyl group is cleaved off and subsequent elimination of acetic acid liberates the reactive intermediate **5**. Crosslinking yields of 70 % are possible with purine rich target RNAs (general product structure **6**).

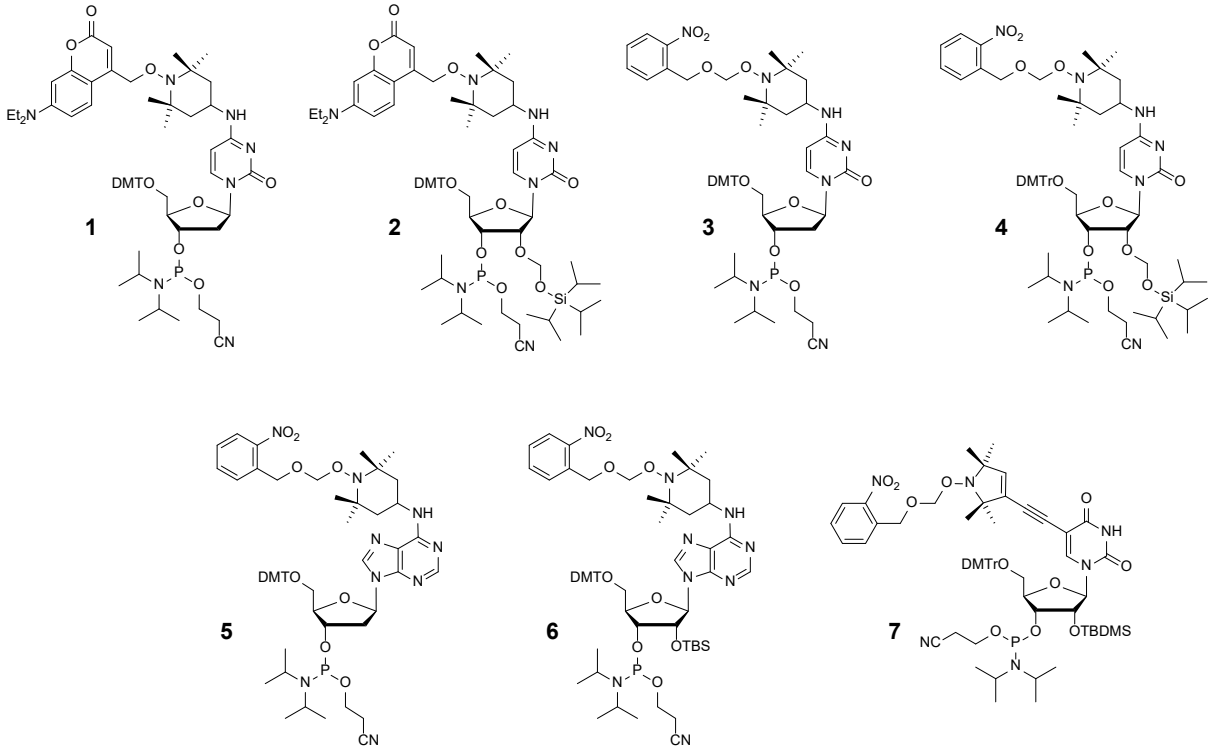
In a second approach, we have investigated the activation of quinone methide precursors **2** – **4** by redox reactions. Due to the presence of glutathione (GSH) at low millimolar concentrations in the cytosol, the disulfide bonds of these conjugates are expected to be cleaved upon cell entry. After elimination of formaldehyde and of acetic acid, the same reactive intermediated **5** would be formed, ready to alkylate complementary RNA (or DNA) strands. The thiol group of GSH, on the other hand, might also quench the quinone methides, a reaction that would prevent the alkylation of RNA. Contrary to such concerns, we could observe similar rates and crosslinking yields around 70 % as found with conjugate **1** (see above) when conjugate **2** was incubated with complementary RNA in the presence of 0.5 mM of GSH. The increased steric hindrance in conjugates **3** – **4**, however, limited rates and yields and high GSH concentrations (10 mM) indeed interfered with RNA crosslinking.

Selected references:

1. Hornung, J.-E., Weinrich, T., and Göbel, M. W. (2022). Directed Crosslinking of RNA by Glutathione-Triggered PNA-Quinone-Methide-Conjugates. Eur. J. Org. Chem., e202200318. https://doi.org/10.1002/ejoc.202200318

2. Hornung, J.-E., Hellwig, N., and Göbel, M. W. (2020). Peptide Nucleic Acid Conjugates of Quinone Methide Precursors Alkylate Ribonucleic Acid after Activation with Light. Bioconjugate Chem. *31*, 639–645. https://doi.org/10.1021/acs.bioconjchem.9b00796

Novel tools for the spin-labeling of DNA and RNA



Nitroxide radicals are valuable spin labels to study the structure and dynamics of nucleic acids by EPR spectroscopy. Although long-living under air and in neutral aqueous solution, conditions typical for the solid-phase synthesis of oligonucleotides and for enzymatic ligation steps can lead to partial or even complete decomposition of nitroxides. To find a general solution of this problem, we have started to develop photolabile protective groups. The coumarin moiety present in phosphoramidites **1** and **2**, for example, can be removed after oligonucleotide synthesis by irradiation at 405 nm. The nitroxide radical is then formed by spontaneous air oxidation of the liberated N-OH group. However, depending on the buffer pH, a significant fraction of the material is permanently reduced to the amine.

A second generation therefore separated the light sensitive part of the protecting group from the labile N-O bond by a methylene spacer: the 2-nitrobenzyloxymethyl group present in phosphoramidites **3** – **7** can be removed without the problem of amine formation. Samples ready for PELDOR studies are obtained by irradiation of the protected oligonucleotides at 365 nm and brief heating to eliminate formaldehyde - avoiding the need of further purification steps. The steric impact of spin labels is known to destabilize folded structures to some extent. This effect is minimized by using the TPA label present in phosphoramidite **7**. However, to avoid misinterpretations of PELDOR data, we recommend in any case to verify the proper fold of spin labeled nucleic acids by secondary structure mapping (e.g. in-line probing in the case of RNA).

Selected references:

1. Kaiser, F., Endeward, B., Collauto, A., Scheffer, U., Prisner, T. F., and Göbel, M. W. (2022). Spin-Labeled Riboswitch Synthesized from a Protected TPA Phosphoramidite Building Block. Chem. Eur. J. *28*, e202201822. https://doi.org/10.1002/chem.202201822

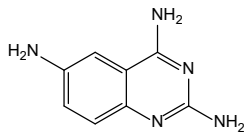
2. Weinrich, T., Jaumann, E. A., Scheffer, U., Prisner, T. F., and Göbel, M. W. (2018). A Cytidine Phosphoramidite with Protected Nitroxide Spin Label: Synthesis of a Full-Length TAR RNA and Investigation by In-Line Probing and EPR Spectroscopy. Chem. Eur. J. *24*, 6202–6207. https://doi.org/10.1002/chem.201800167

3. Weinrich, T., Jaumann, E. A., Scheffer, U. M., Prisner, T. F., and Göbel, M. W. (2018). Phosphoramidite building blocks with protected nitroxides for the synthesis of spin-labeled DNA and RNA. Beilstein J. Org. Chem. *14*, 1563–156. https://doi.org/10.3762/bjoc.14.133

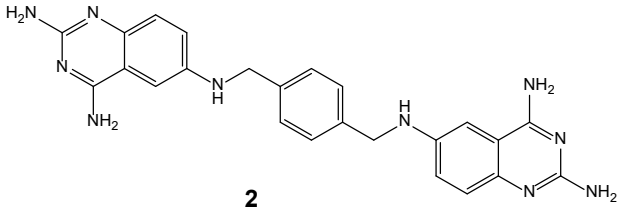
4. Weinrich, T., Gränz, M., Grünewald, C., Prisner, T. F., and Göbel, M. W. (2017). Synthesis of a Cytidine Phosphoramidite with Protected Nitroxide Spin Label for EPR Experiments with RNA. Eur. J. Org. Chem., 491–496. https://doi.org/10.1002/ejoc.201601174

5. Seven, I., Weinrich, T., Gränz, M., Grünewald, C., Brüß, S., Krstić, I., Prisner, T. F., Heckel, A., and Göbel, M. W. (2014). Photolabile Protecting Groups for Nitroxide Spin Labels. Eur. J. Org. Chem., 4037–4043. https://doi.org/10.1002/ejoc.201301692

Small molecule ligands acting on viral RNAs



1



2

TAR, the transactivation response element of HIV, is a stable stem-loop structure of 59 ribonucleotides located at the 5' end of all viral transcripts. TAR binds the arginine rich viral protein Tat in a way that mimics the 7SK complex with HEXIM1. The Tat-TAR complex recruits the human positive transcription elongation factor b (P-TEFb) to viral mRNAs, causing a phosphorylation of RNA polymerase II and thus a dramatic upregulation of viral transcription. Therefore, the search for small molecules binding to TAR and blocking its function has been a long-standing field of research. Our group has investigated short cationic peptides containing synthetic heteroaromatic amino acids. Some of these peptides show significant Tat-TAR inhibition.

In a different approach, we have combined small ligand fragments into simple heteroaromatic molecules. Within few iteration steps, the IC_{50} value in a Tat-TAR displacement assay could be improved from >20 mM to 40 µM (compound **1**). Subsequent NMR studies with quinazoline **1** demonstrated the presence of two high affinity binding sites within TAR. We then connected two copies of **1** by spacers of different length and rigidity (unpublished work). With dimer **2** a sharp peak of activity was found with an IC_{50} value of 150 nM.

The lessons learned from HIV-TAR are presently applied to a search for ligands of conserved RNA elements from SARS-CoV-2.

Selected references:

1. Sreeramulu, S., Richter, C., Berg, H., Wirtz Martin, M. A., Ceylan, B., Matzel, T., Adam, J., Altincekic, N., Azzaoui, K., and Bains, J. K., Blommers, M. J. J., Ferner, J., Fürtig, B., Göbel, M., Grün, J. T., Hengesbach, M., Hohmann, K. F., Hymon, D., Knezic, B., Martins, J., Mertinkus, K. R., Niesteruk, A., Peter, S. A., Pyper, D. J., Qureshi, N. S., Scheffer, U., Schlundt, A., Schnieders, R., Stirnal, E., Sudakov, A., Tröster, A., Vögele, J., Wacker, A., Weigand, J. E., Wirmer-Bartoschek, J., Wöhnert, J., and Schwalbe, H. (2021). Exploring the druggability of conserved RNA regulatory elements in the SARS-CoV-2 genome. Angew. Chem. Int. Ed. *60*, 19191–19200. https://doi.org/10.1002/anie.202103693

2. Zeiger, M., Stark, S., Kalden, E., Ackermann, B., Ferner, J., Scheffer, U., Shoja-Bazargani, F., Erdel, V., Schwalbe, H., and Göbel, M. W. (2014). Fragment based search for small molecule inhibitors of HIV-1 Tat-TAR. Bioorg. Med. Chem. Lett. *24*, 5576–5580. https://doi.org/10.1016/j.bmcl.2014.11.004

3. Ferner, J., Suhartono, M., Breitung, S., Jonker, H. R. A., Hennig, M., Wöhnert, J., Göbel, M., and Schwalbe, H. (2009). Structures of HIV TAR RNA-Ligand Complexes Reveal Higher Binding Stoichiometries. ChemBioChem *10,* 1490–1494. https://doi.org/10.1002/cbic.200900220

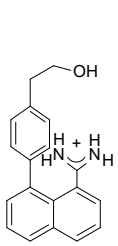
4. Morgner, N., Barth, H.-D., Brutschy, B., Scheffer, U., Breitung, S., and Göbel, M. (2008). Binding sites of the viral RNA element TAR and of TAR mutants for various peptide ligands,

probed with LILBID: A new laser mass spectrometry. J. Am. Soc. Mass. Spectrom. *19*, 1600–1611.
https://doi.org/10.1016/j.jasms.2008.07.001

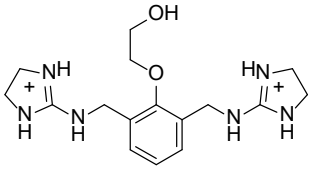
5. Suhartono, M., Weidlich, M., Stein, T., Karas, M., Dürner, G., and Göbel, M. W. (2008). Synthesis of non-natural aromatic alpha -amino acids by a Heck reaction. Eur. J. Org. Chem., 1608–1614.
https://doi.org/10.1002/ejoc.200701124

6. Ludwig, V., Krebs, A., Stoll, M., Dietrich, U., Ferner, J., Schwalbe, H., Scheffer, U., Dürner, G., and Göbel, M. W. (2007). Tripeptides from synthetic amino acids block the Tat-TAR association and slow down HIV spread in cell cultures. ChemBioChem *8*, 1850–1856.
https://doi.org/10.1002/cbic.200700232

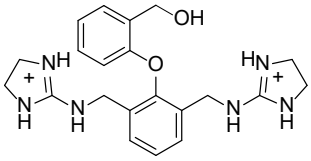
Anion receptors with nucleophilic side chains: Cleavage of phosphodiesters and of DNA



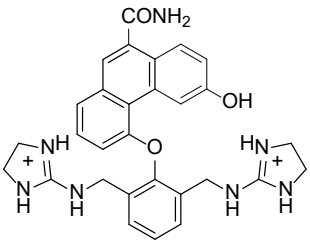
1



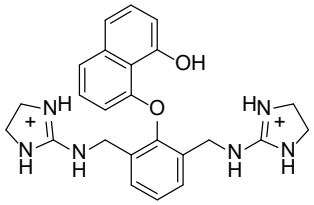
2



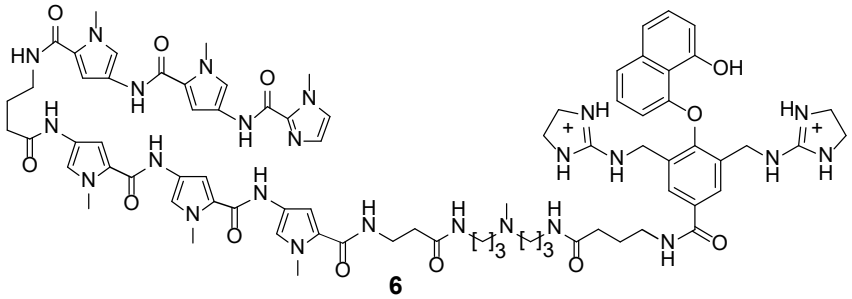
3



4



5



6

Phosphodiester anions are known for their exceptional kinetic stability. Phosphoryl transfer reactions catalyzed by enzymes, on the other hand, can be very fast – rate increases up to 17 orders of magnitude are known. The active sites of such enzymes typically contain positively charged amino acids and metal ions as electrophiles, water or tyrosine side chains as nucleophiles. None of these groups are very potent by themselves. The catalytic power of enzymes emerges from the specific arrangement of functional groups in the active site. Amidinium alcohol **1** was designed as a simplified model of phosphodiesterase enzymes. In dipolar aprotic solvents, the protonated amidine forms ion pairs with catechol cyclic phosphate. In comparison with uncharged alcohols, the phosphorylation of the OH group of **1** is accelerated by a factor of 2700. This rate effect is explained in part by the proximity of reacting groups, in part by electrophilic activation of the phosphate within the ion pair complex. Much larger effects (10^6 to 10^7fold) are observed with the dicationic alcohol **2**. However, the kinetic advantage shrinks with less reactive substrates and phosphorylation occurs at the guanidine nitrogens preferentially. Systematic variation of the hydroxy side chain (e.g. **3** and **4**) finally led us to alcohol **5**. This compound reacts with several less activated phosphodiesters with high rates and at oxygen selectively. Bisguanidinium alcohol **5** at millimolar concentration also cleaves plasmid DNA in aqueous buffer. When attached to a minor groove binding hairpin polyamide, plasmid cleavage above background by the resulting conjugate **6** is observed at concentrations as low as 78 nM (corresponding to 3-4 copies of conjugate **6** per plasmid). The mechanism of cleavage, however, is not yet fully characterized.

Selected references:

1. Wirth-Hamdoune, D., Ullrich, S., Scheffer, U., Radanović, T., Dürner, G., and Göbel, M. W. (2016). A Bis(guanidinium)alcohol Attached to a Hairpin Polyamide: Synthesis, DNA Binding, and Plasmid Cleavage. ChemBioChem *17*, 506–514. https://doi.org/10.1002/cbic.201500566

2. Ullrich, S., Nazir, Z., Büsing, A., Scheffer, U., Wirth, D., Bats, J. W., Dürner, G., and Göbel, M. W. (2011). Cleavage of Phosphodiesters and of DNA by a Bis(guanidinium)naphthol Acting as

a Metal-Free Anion Receptors. ChemBioChem *12,* 1223–1229. https://doi.org/10.1002/cbic.201100022

3. Muche, M.-S., Kamalaprija, P., and Göbel, M. W. (1997). Reaction cascade in the supramolecular phosphorylation of a bis(guanidinium) diol. Tetrahedron Lett. *38*, 2923–2926. https://doi.org/10.1016/S0040-4039(97)00513-3

4. Muche, M.-S., and Göbel, M. W. (1996). Bis(guanidinium) alcohols as models of staphylococcal nuclease: substrate binding through ion pair complexes and fast phosphoryl transfer reactions. Angew. Chem. Int. Ed. *35*, 2126–2129. https://doi.org/10.1002/anie.199621261

5. Reckenbeil, D., Bats, J. W., Dürner, G., and Göbel, M. W. (1994). Supramolecular phosphorylation of cationic alcohols with a 3-arylindole-4-carboxyamidine framework. Liebigs Ann. Chem., 1219–1229. https://doi.org/10.1002/jlac.199419941213

6. Müller, G., Dürner, G., Bats, J. W., and Göbel, M. W. (1994). Supramolecular phosphoryl transfer reactions mediated by amidinium phosphate ion-pair complexes. Liebigs Ann. Chem., 1075–1092. https://doi.org/10.1002/jlac.199419941106

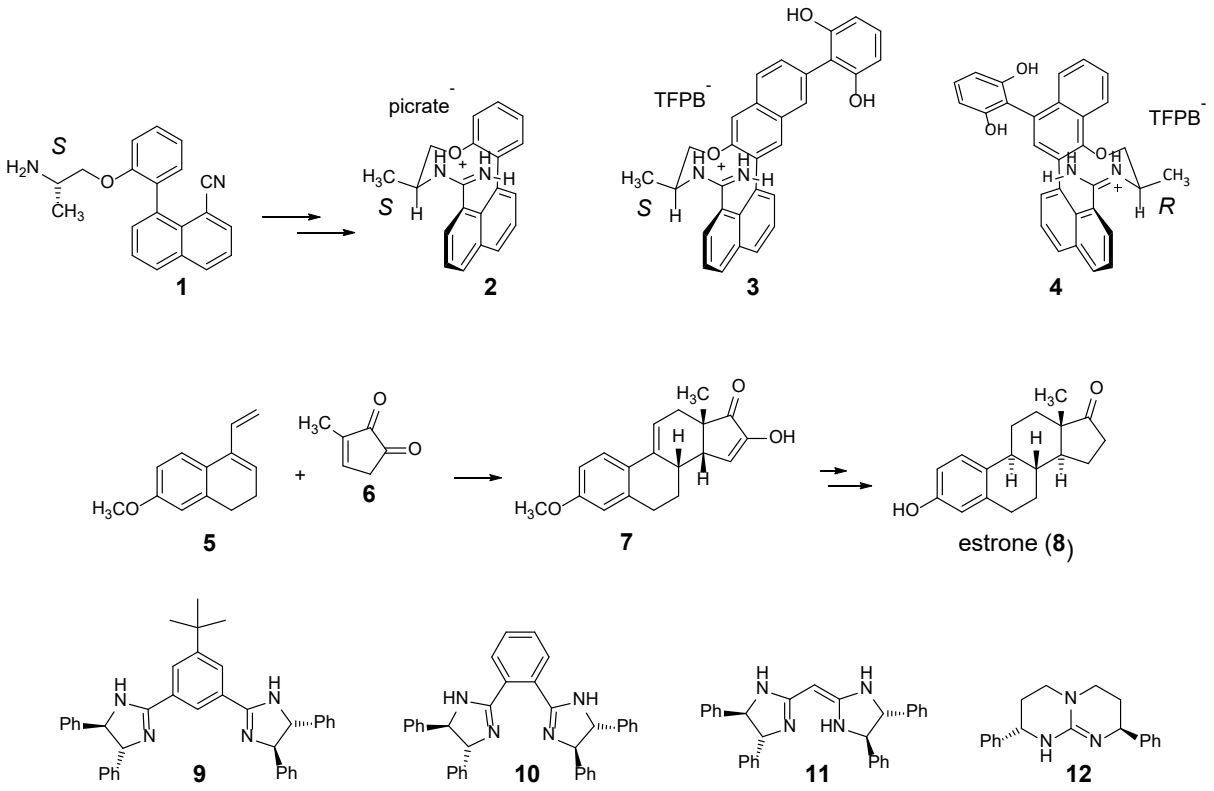
7. Gross, R., Bats, J. W., and Göbel, M. W. (1994). trans-Decalin as a spacer for axially configurated diamines: preparation of a phosphate binding bis(guanidinium) compound. Liebigs Ann. Chem., 205–210. https://doi.org/10.1002/jlac.199419940215

8. Gross, R., Dürner, G., and Göbel, M. W. (1994). Acceleration of substitution reactions of a phosphoric acid diester by bis(guanidinium) compounds. Liebigs Ann. Chem., 49–58. https://doi.org/10.1002/jlac.199419940110

9. Göbel, M. W., Bats, J. W., and Dürner, G. (1992). En Route to Synthetic Phosphodiesterases: Supramolecular Phosphoryl-Transfer Mediated by Amidinium–Phosphate Contact Ion-Pairs. Angew. Chem. Int. Ed. *31*, 207–209. https://doi.org/10.1002/anie.199202071

Previous research activities of the group:

Enantioselective organocatalysis



Axially chiral amidines. The rotation around the biaryl axis of amino nitrile **1** is slow and thus two diastereomeric rotamers can be separated by HPLC at room temperature. At elevated temperature the interconversion of rotamers becomes fast. When the amino group of **1** is deprotonated at high temperature, a nucleophilic addition to the nitrile occurs, forming an amidine which can be isolated in form of picrate **2**. Interestingly, both rotamers react in a stereoconvergent way: a single isomer of **2** is produced and the *S*-configurated stereocenter controls the configuration of the chiral axes and planes with perfect selectivity. The reason for this is the chair-like conformation of the newly formed ten-membered ring in structure **2** and the equatorial position of the methyl group. Cyclization of the other rotamer of **1** would force the methyl group into an axial position. This is not possible for steric reasons. The same principle also governs the cyclization of other axially-chiral amidines such as compounds **3** and **4**.

A tailor-made catalyst for the synthesis of (+)-estrone (8). The CD spectrum of amidinium picrate **2** shows a strong induced band around 420 nm demonstrating the chiral perturbation of the achiral picrate ion in the host-guest complex. Similar effects occur with the yellow-colored diketone **6**, a building block for estrone. Catalyzed by traditional chiral Lewis acids, the Diels-Alder reaction of Dane's diene **5** and diketone **6** had been used previously as a key step in the total synthesis by Quinkert and coworkers. As early as in 1992 we believed that binding of **6** to a chiral hydrogen bond donor should be an alternative approach to control cycloadditions enantioselectively. Since then, we have been active to develop amidinium catalysts for the Diels-Alder reaction of compounds **5** and **6**. After exchange of picrate against non-coordinating anions (TFPB$^-$: tetrakis(3,5-bis(trifluoromethyl)phenyl)-borate), the axially chiral amidinium ion **2** indeed accelerates the cycloaddition and induces low levels of enantioselectivity. To improve the selectivity via three-point recognition, amidinium ions with additional H-bond donors have been synthesized (e.g. **3** and **4**). Best results are obtained with 15 mol%

of catalyst **4** in CH_2Cl_2 at -30 °C. Estrone precursor **7** is formed in 91 % yield (81 % ee). Further steps and a single recrystallization give access to (+)-estrone (99,9 % ee) in a total yield of 24 % based on diketone **6**.

Chiral bisamidines and guanidines. Over the years, some other chiral bisamidines **9** – **11** and the guanidine **12** have been synthesized and tested as catalysts for enantioselective transformations.

Selected references:

1. Goldberg, M., Nakajima, M., Bolte, M., and Göbel, M. W. (2022). Facile conversion of 1,2-dicyanobenzene into chiral bisamidines. Arkivoc 2021, 225–238. https://doi.org/10.24820/ark.5550190.p011.722

2. Goldberg, M., Sartakov, D., Bats, J. W., Bolte, M., and Göbel, M. W. (2016). A chiral analog of the bicyclic guanidine TBD. Synthesis, structure and Brønsted base catalysis. Beilstein J. Org. Chem. *12*, 1870–1876. https://doi.org/10.3762/bjoc.12.176

3. Weimar, M., Dürner, G., Bats, J. W., and Göbel, M. W. (2010). Enantioselective Synthesis of (+)-Estrone Exploiting a Hydrogen Bond-Promoted Diels-Alder Reaction. J. Org. Chem. *75*, 2718–2721. https://doi.org/10.1021/jo100053j

4. Akalay, D., Dürner, G., and Göbel, M. W. (2008). A first case of asymmetric catalysis induced by metal-free bisoxazolines. Eur. J. Org. Chem., 2365–2368. https://doi.org/10.1002/ejoc.200800179

5. Akalay, D., Dürner, G., Bats, J. W., and Göbel, M. W. (2008). C2-symmetric bisamidines: chiral Brønsted bases catalysing the Diels-Alder reaction of anthrones. Beilstein J. Org. Chem. *4*, No. 28. https://doi.org/10.3762/bjoc.4.28

6. Akalay, D., Dürner, G., Bats, J. W., Bolte, M., and Göbel, M. W. (2007). Synthesis of C_2-Symmetric Bisamidines: A New Type of Chiral Metal-Free Lewis Acid Analogue Interacting with Carbonyl Groups. J. Org. Chem. *72*, 5618–5624. https://doi.org/10.1021/jo070534j

7. Tsogoeva, S. B., Dürner, G., Bolte, M., and Göbel, M. W. (2003). A C_2-chiral bis(amidinium) catalyst for a Diels-Alder reaction constituting the key step of the Quinkert-Dane estrone synthesis. Eur. J. Org. Chem., 1661–1664. https://doi.org/10.1002/ejoc.200200635

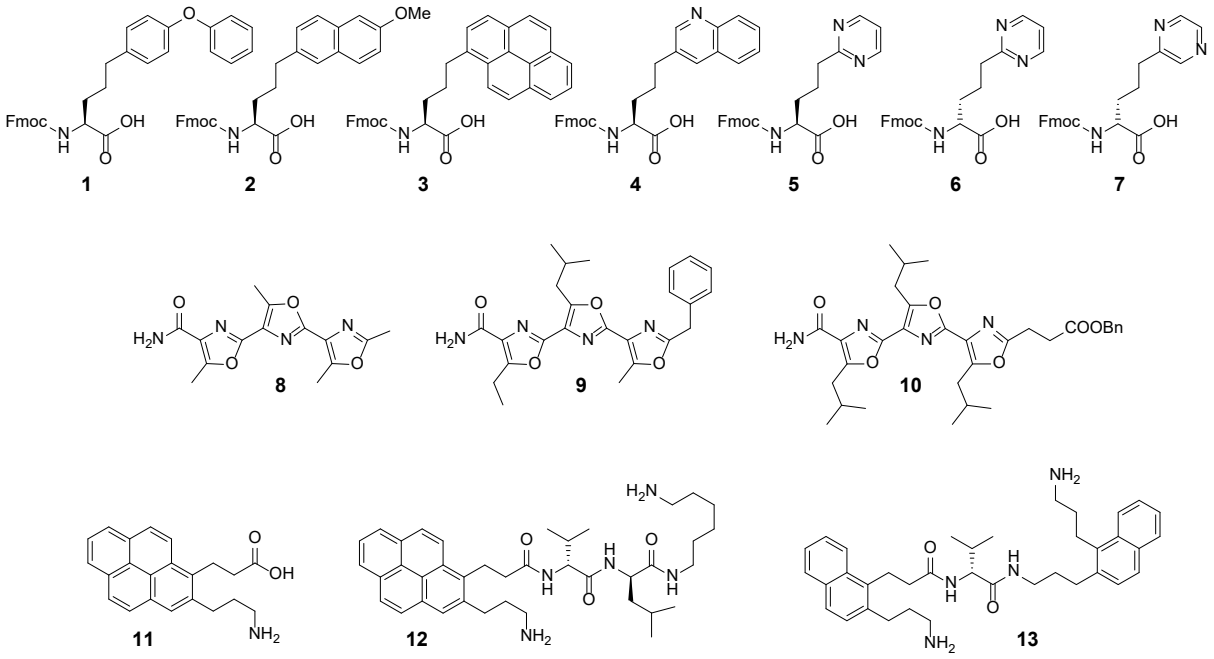
8. Schuster, T., Bauch, M., Dürner, G., and Göbel, M. W. (2000). Axially Chiral Amidinium Ions as Inducers of Enantioselectivity in Diels-Alder Reactions. Org. Lett. *2*, 179–181. https://doi.org/10.1021/ol991276i

9. Schuster, T., Kurz, M., and Göbel, M. W. (2000). Catalysis of a Diels-Alder Reaction by Amidinium Ions. J. Org. Chem. *65*, 1697–1701. https://doi.org/10.1021/jo991372x

10. Schuster, T., and Göbel, M. W. (1999). Synthesis of a chiral receptor molecule with converging amidinium and hydroxy groups. Synlett, 966–968. https://doi.org/10.1055/s-1999-3095

11. Lehr, S., Schütz, K., Bauch, M., and Göbel, M. W. (1994). Axially Chiral Amidinium Ions with a Biaryl Skeleton: A New Class of Structure in Host–Guest Chemistry. Angew. Chem. Int. Ed. *33*, 984–986. https://doi.org/10.1002/anie.199409841

Synthetic amino acids and peptidomimetics



In a project related to peptidic RNA ligands (see above), we synthesized a variety of synthetic α-amino acids with aromatic and heteroaromatic side chains. The *S*-configurated compounds **1** – **5**, for example, were prepared from L-glutamic acid with retention of configuration. Analogous methods to obtain compounds with a C_2 linker started from L-aspartic acid or L-methionine. The *R*-configurated amino acids like **6** and **7** could be prepared from *R*-allyl glycine via Myers alkylation.

Compounds designed to mimic α-peptide helices are interesting candidates to block protein-protein interactions. A well-studied class of helix mimetics are terphenyls decorated with appropriate side chains. As a structural alternative to terphenyls we have developed the related teroxazoles. The synthesis of compounds **8** – **10** started from serine, threonine or threonine derivatives with extended side chains. The amino acids were then assembled by solid-phase peptide synthesis, and ring closure was achieved by a Robinson-Gabriel oxazole synthesis.

The Catellani reaction is a versatile three-component reaction catalyzed by palladium and norbornene. We have applied this reaction to synthesize a number of non-natural amino acids like **11**, building blocks used to prepare the peptidomimetics **12**, **13** and others. Such peptidomimetics interfere with the aggregation of Aβ towards amyloid plaques, a phenomenon involved in the development of Alzheimer's disease.

Selected references:

1. Stark, T., Lieblein, T., Pohland, M., Kalden, E., Freund, P., Zangl, R., Grewal, R., Heilemann, M., Eckert, G. P., Morgner, N., and Göbel, M. W. (2017). Peptidomimetics that inhibit and partially reverse the Aggregation of Aβ1-42. Biochemistry *56*, 4840–4849. https://doi.org/10.1021/acs.biochem.7b00223

2. Stark, T., Suhartono, M., Göbel, M. W., and Lautens, M. (2013). A Palladium-Catalyzed Domino Reaction as Key Step for the Synthesis of Functionalized Aromatic Amino Acids. Synlett *24*, 2730–2734. https://doi.org/10.1055/s-0033-1339892

3. Cernescu, M., Stark, T., Kalden, E., Kurz, C., Leuner, K., Deller, T., Göbel, M., Eckert, G. P., and Brutschy, B. (2012). Laser-Induced Liquid Bead Ion Desorption Mass Spectrometry: An Approach to Precisely Monitor the Oligomerization of the β-Amyloid Peptide. Anal. Chem. *84*, 5276–5284. https://doi.org/10.1021/ac300258m

4. Pinto Gomes, C., Metz, A., Bats, J. W., Gohlke, H., and Göbel, M. W. (2012). Modular Solid-Phase Synthesis of Teroxazoles as a Class of α-Helix Mimetics. Eur. J. Org. Chem., 3270–3277. https://doi.org/10.1002/ejoc.201200339

5. Suhartono, M., Schneider, A. E., Dürner, G., and Göbel, M. W. (2010). Synthetic aromatic amino acids from a Negishi cross-coupling reaction. Synthesis, 293–303. https://doi.org/10.1055/s-0029-1217124

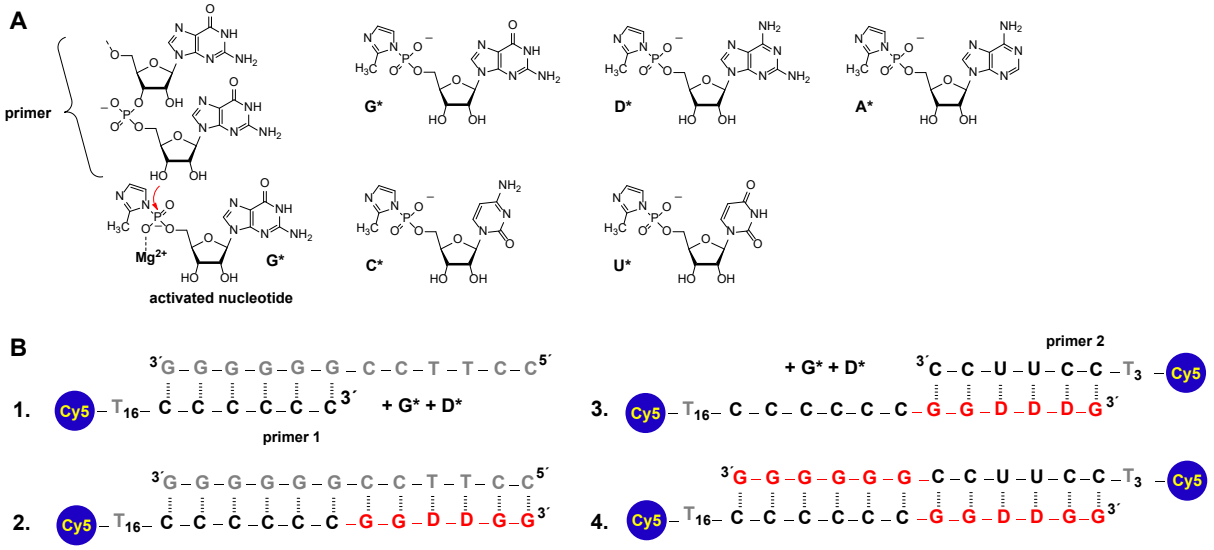
6. Breitung, S. T., Lopez, J. J., Dürner, G., Glaubitz, C., Göbel, M. W., and Suhartono, M. (2008). A practical synthesis of the $^{13}C/^{15}N$-labeled tripeptide N-Formyl-Met-Leu-Phe, useful as a reference in solid-state NMR spectroscopy. Beilstein J. Org. Chem. *4*, No. 35. https://doi.org/10.3762/bjoc.4.35

7. Suhartono, M., Weidlich, M., Stein, T., Karas, M., Dürner, G., and Göbel, M. W. (2008). Synthesis of non-natural aromatic alpha -amino acids by a Heck reaction. Eur. J. Org. Chem., 1608–1614. https://doi.org/10.1002/ejoc.200701124

8. Ludwig, V., Krebs, A., Stoll, M., Dietrich, U., Ferner, J., Schwalbe, H., Scheffer, U., Dürner, G., and Göbel, M. W. (2007). Tripeptides from synthetic amino acids block the Tat-TAR association and slow down HIV spread in cell cultures. Chembiochem *8*, 1850–1856. https://doi.org/10.1002/cbic.200700232

9. Krebs, A., Ludwig, V., Pfizer, J., Dürner, G., and Göbel, M. W. (2004). Enantioselective synthesis of non-natural aromatic alpha-amino acids. Chemistry *10*, 544–553. https://doi.org/10.1002/chem.200305421

Template controlled oligomerization of RNA



A. Classical experiments by Leslie Orgel have shown that template-controlled primer extension is possible with ribonucleoside 5'-phosphates activated by 2-methyl imidazole (e.g. **G***). 3',5' bonds are formed preferentially in this enzyme-free reaction. Although surprisingly effective, spontaneous self-replication and Darwinian evolution have never been observed in such experiments. Some years ago, we have inspected the Orgel system and found solutions to overcome some of the obstacles.

1. The primer-template duplex should adopt the A-helix conformation. Thus, at least one of the strands, primer or template, should consist of ribonucleotides.

2. High concentrations of Na^+, which have been used previously, do not accelerate the primer extension. In contrast, they induce quadruplex formation of G-rich strands. At low Na^+ concentrations, the assembly of **C*** on oligo-G templates works quite effectively.

3. Replacement of adenosine (**A***) by the corresponding diamino purine analog (**D***) impressively improves the primer extension opposite to U or T sites in the template as a result of increased base pair stability.

B. Taking advantage of our experimental improvements, we were able to carry out an PCR-inspired experiment: Step 1. The dye-labeled RNA primer 1 is incubated with **G*** and **D*** in presence of a DNA template (RNA: black letters; DNA: grey; freshly assembled RNA: red). Step 2. Primer 1 has been extended by the sequence GGDDGG (red). Step 3. After isolation, the red part of the extended primer now serves as a binding site for the orthogonal primer 2. Chain extension starts in the presence of the activated monomers **G*** and **D***. Step 4. Full extension of primer 2 completes the replication of the template sequence introduced in step 1. In contrast to PCR, however, a purification of the extended primer 1 is indispensable for the success of the reverse reaction in steps 3 and 4. Although mixed sequences of G and D are easily obtained in Orgel-type oligomerization experiments, the yield drops with pyrimidine monomers (**C***) and each change between purines and pyrimidines has a deleterious effect on chain extension.

Selected references:

1. Hey, M., Hartel, C., and Göbel, M. W. (2003). Nonenzymatic oligomerization of ribonucleotides: Towards in vitro selection experiments. Helv. Chim. Acta *86*, 844–854. https://doi.org/10.1002/hlca.200390084

2. Hartel, C., and Göbel, M. W. (2000). Substitution of adenine by purine-2,6-diamine improves the nonenzymatic oligomerization of ribonucleotides on templates containing thymidine. Helv. Chim. Acta *83*, 2541–2549. https://doi.org/10.1002/1522-2675(20000906)83:9%3C2541::AID-HLCA2541%3E3.0.CO;2-8

3. Kurz, M., Göbel, K., Hartel, C., and Göbel, M. W. (1998). Acridine-labeled primers as tools for the study of nonenzymatic RNA oligomerization. Helv. Chim. Acta *81*, 1156–1180. https://doi.org/10.1002/hlca.19980810528

4. Kurz, M., Göbel, K., Hartel, C., and Göbel, M. W. (1997). Nonenzymatic oligomerization of ribonucleotides on guanosine-rich templates: suppression of the self-pairing of guanosine. Angew. Chem. Int. Ed. *36*, 842–845. https://doi.org/10.1002/anie.199708421

5. Schütz, K., Kurz, M., and Göbel, M. W. (1995). Synthesis of oligodeoxyribonucleotides labeled with a novel type of chemically stable acridine dye. Tetrahedron Lett. *36*, 8407–8410. https://doi.org/10.1016/0040-4039(95)01801-N