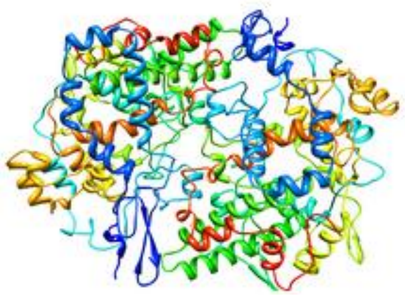
# Linkerology®

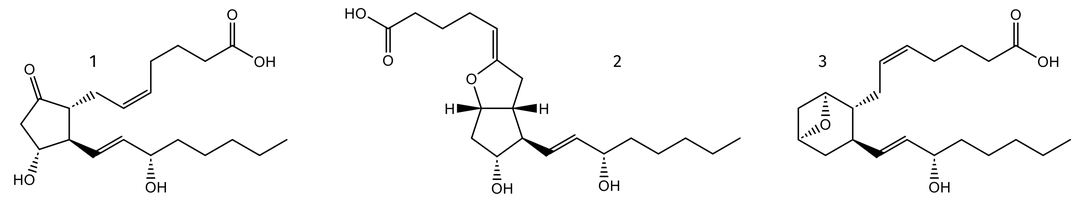
## 2023 # 09 – Multiple Conjugation for Combination Therapy

Examples how pain treatment drugs can be decorated with different (self-immolative) linkers

### Biological background of pain



Prostanoids, like thromboxane and prostaglandins, like prostacyclin are being synthesized by **cyclooxygenase** (**COX**) [EC 1.14.99.1], a prostaglandin-endoperoxide synthase (PTGS) and are responsible for feeling pain and inflammation.
COX, therefore, is a common target for pain relief and anti-inflammatory drugs, as its function triggers feelings like, headache, toothache, migraine, or larger pain in case of severe trauma.

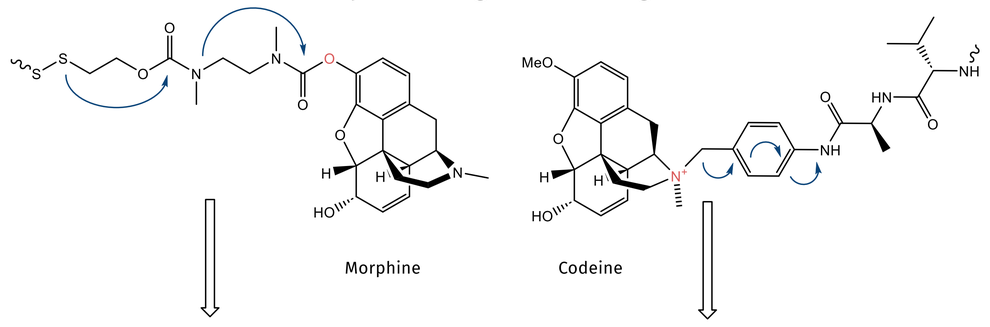


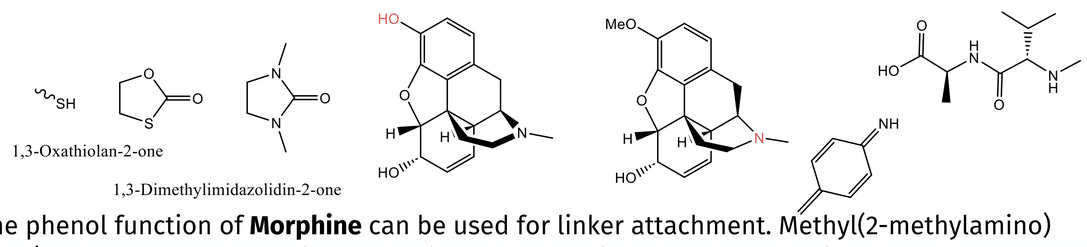

Prostaglandin (1) is being created where tissue damage or infection occurs and is causing inflammation and pain.

Prostacyclin (2) removes blood clots and dilates blood vessels to increase blood flow.

Thromboxane (3) triggers the clotting of platelets and constricts blood vessels to decrease blood loss.

### Examples of cargo-linker design

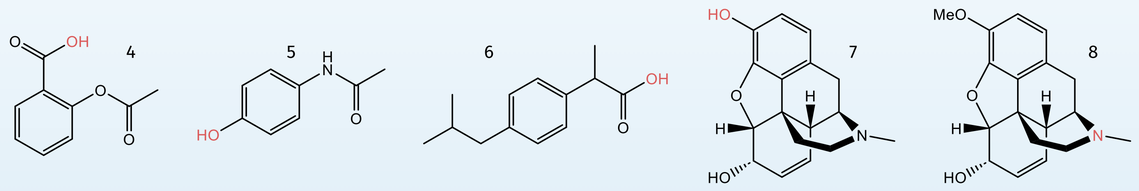



The phenol function of **Morphine** can be used for linker attachment. Methyl(2-methylamino) ethyl)carbamate can be conjugated to it and further linked to the reductive cleavable dithioethylcarbamate.

In **Codeine** the phenol function, which is free in Morphine, is methylated and blocked for further conjugation. The tertiary amino function can be alkylated by a PAB linker, which can further be linked to valyl-alanine.

### Conventional drugs treating pain



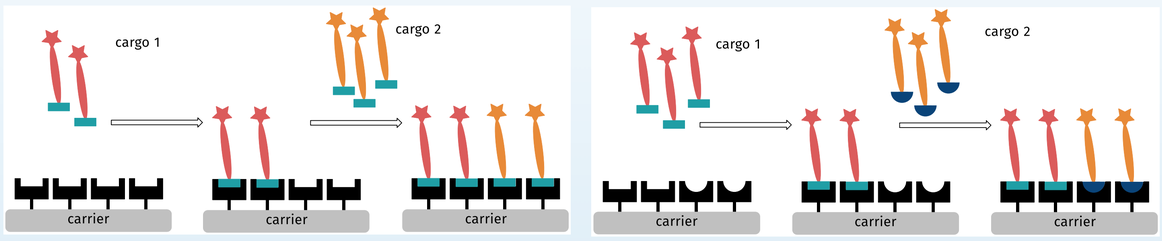

**Aspirin®** (4, acetylsalicylic acid), is used to reduce pain, fever, and/or inflammation, and as an antithrombotic. It carries a free carboxylic acid function, which can be used for linker attachment.

**Paracetamol®** (5, *p*-hydroxyacetanilide) is a non-opioid analgesic agent used to treat fever and mild to moderate pain. It is commonly used just as Aspirin® and bears a phenol group to conjugate a linker.

**Ibuprofen®** (6) is a nonsteroidal anti-inflammatory drug (NSAID) that is used to relieve pain, fever, and inflammation. This includes painful menstrual periods, migraines, and rheumatoid arthritis. Similar, like Aspirin® it carries a free carboxylic acid and no other functional group, an ideal constellation for linker attachment.

**Morphine** (7) and **Codeine** (8) are both strong opiate found in natural opium. They are mainly used as an analgesic in severe cases for both acute pain (myocardial infarction, kidney stones, etc.) and chronic pain. It acts directly on the central nervous system (CNS) to induce analgesia and alter perception and emotional response to pain. Physical and psychological dependence and tolerance may develop with repeated administration. Therefore, this class of compounds falls under The Controlled Drugs and Substances Act (CDSA)!

### Strategies for multiple carrier-cargo conjugation



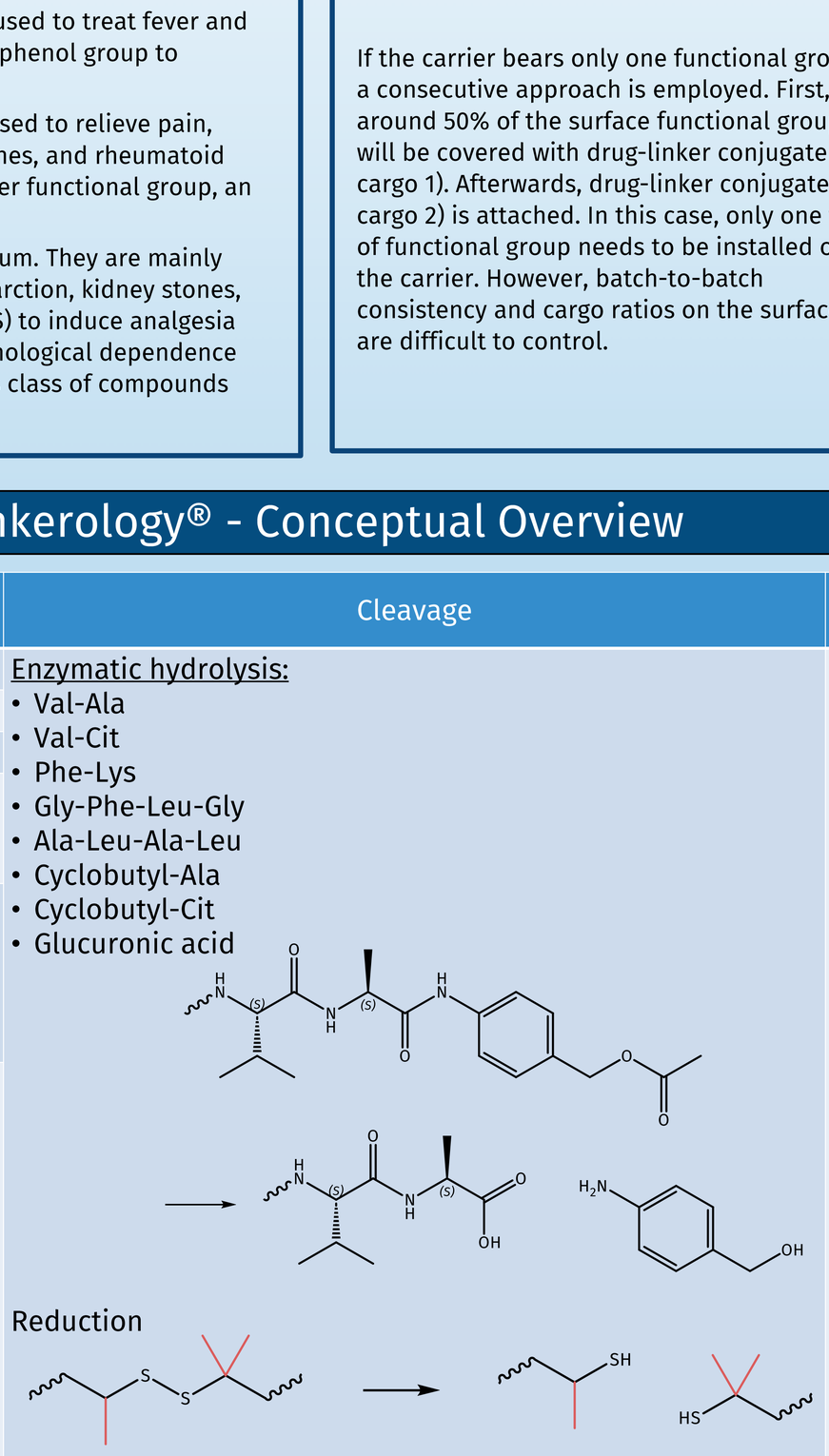
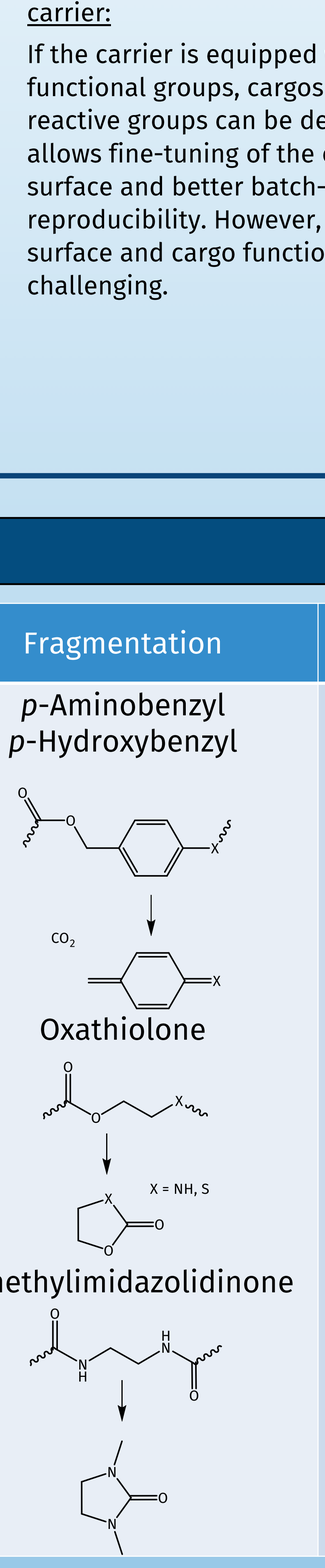
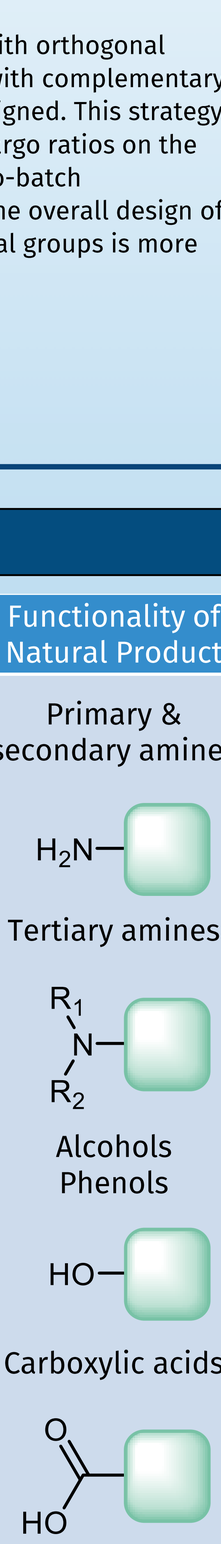

<u>One functional group per carrier:</u>

If the carrier bears only one functional group, a consecutive approach is employed. First, around 50% of the surface functional groups will be covered with drug-linker conjugate 1 (= cargo 1). Afterwards, drug-linker conjugate 2 (= cargo 2) is attached. In this case, only one type of functional group needs to be installed on the carrier. However, batch-to-batch consistency and cargo ratios on the surface are difficult to control.

<u>Several orthogonal functional groups per carrier:</u>

If the carrier is equipped with orthogonal functional groups, cargos with complementary reactive groups can be designed. This strategy allows fine-tuning of the cargo ratios on the surface and better batch-to-batch reproducibility. However, the overall design of surface and cargo functional groups is more challenging.

## Linkerology® - Conceptual Overview

| Carrier | Surface Treatment & Conjugation Chemistry | Cleavage | Fragmentation | Functionality of Natural Product |
|---|---|---|---|---|
| Metal surface | Affinity of sulfur to gold and silver | <u>Enzymatic hydrolysis:</u><br>• Val-Ala<br>• Val-Cit<br>• Phe-Lys<br>• Gly-Phe-Leu-Gly<br>• Ala-Leu-Ala-Leu<br>• Cyclobutyl-Ala<br>• Cyclobutyl-Cit<br>• Glucuronic acid | *p*-Aminobenzyl<br>*p*-Hydroxybenzyl | Primary & secondary amines |
| Metal oxide | Chelat formation | | | |
| Silicates | Affinity of silicon and oxygen | | | |
| <u>Carbon:</u><br>• Nanotubes<br>• Fullerenes | Nitrenen addition via photoactivation of perfluoroarylazides | | | |
| <u>Plastic polymers:</u><br>• Teflon<br>• Polyethylene<br>• Polystyrene<br>• Latex | Ammonia or acrylic acid plasma followed by amide bond formation | | | |
| <u>Biopolymers:</u><br>• Peptides<br>• Proteins<br>• Antibodies<br>• Single Chain<br>• Nanobodies<br>• Camelides<br>• Oligonucleotides<br>• Aptamers | Thioether formation with maleimide<br>Disulfide bond formation<br>Acylation of Amines<br>His-Tag acylation<br>Click conjugation (CuCAAC, SPAAC, IEDDA)<br>Enzyme supported conjugation:<br>HaloTag®<br>CLIP-Tag™<br>SNAP-Tag®<br>Sequence dependent conjugation (Sortase) |  <br>Reduction |  <br>Oxathiolone<br>Dimethylimidazolidinone |  <br>Tertiary amines<br>Alcohols<br>Phenols<br>Carboxylic acids |

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