Covalent Coupling Procedure on SiMAG-Amine by Mannich Condensation

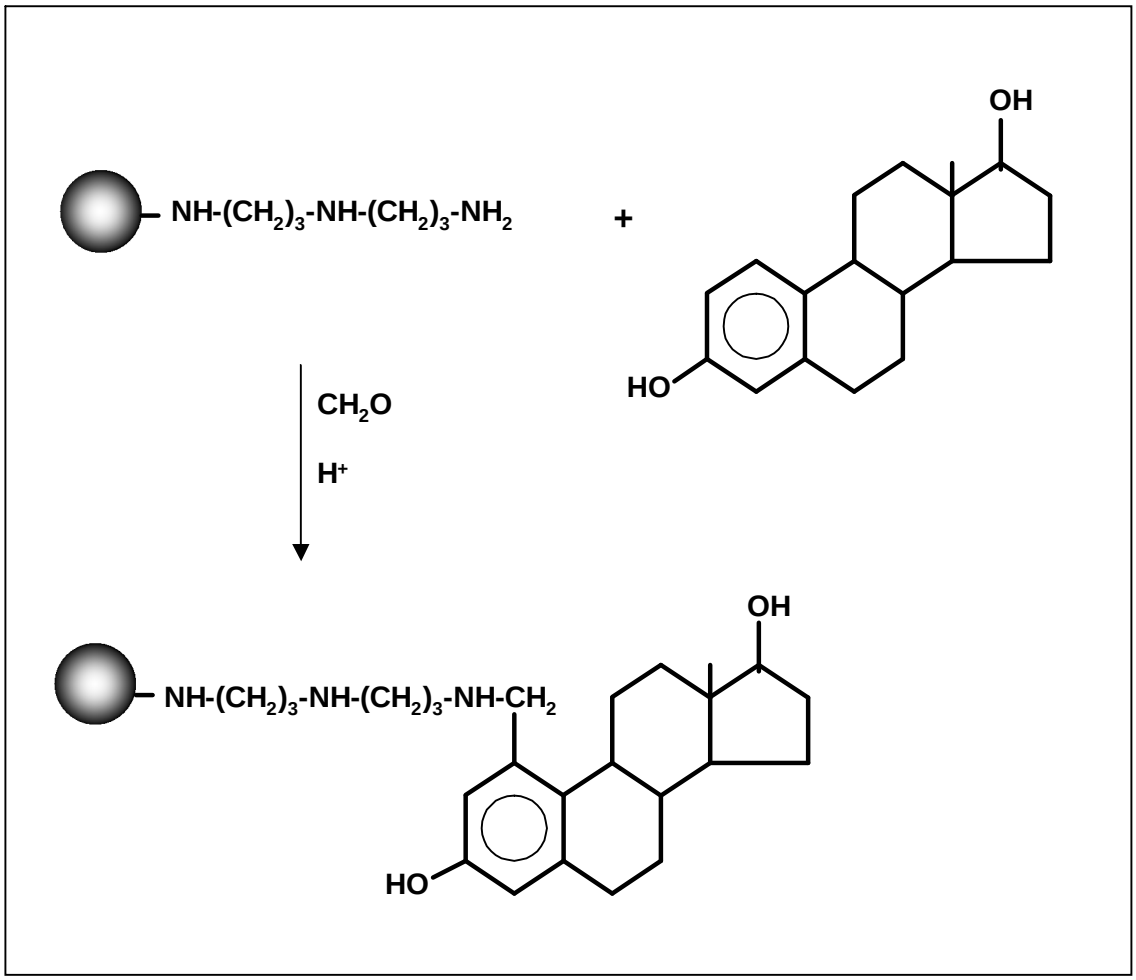
Introduction:

This procedure describes the immobilization of compounds via aromatic ring to **SiMAG-Amine** by the Mannich reaction.

The Mannich reaction is useful for the coupling of compounds such as drugs, steroidal compounds, dyes, or other organic molecules which do not contain reactive components, e.g. primary amines, carboxylic acids, aldehydes or sulfhydryl groups.

The ligands are coupled by condensation with formaldehyde to terminal amine groups of the beads.

The coupling via Mannich reaction forms very stable covalent bonds which are useful for most affinity separations.



Equipment and reagents:

- **SiMAG-Amine**
- **Formaldehyde 37 %**
- **0.1 M 2-(N-Morpholino)ethanesulfonic acid (MES), pH 4.7**
- **Blocking & Storage Buffer:** PBS, 0.1 % BSA, 0.05 % sodium azide
- **Magnetic Separator** (**e.g. MagnetoPURE,** Product Number: MP-10)

Technical Note:

- If the ligand is not soluble in plain water or aqueous buffer, the reaction can be carried out in solutions containing up to 50 % ethanol.
- For antibodies or proteins we recommend to use a minimum amount of 50 µg antibody/protein per 10 mg **SiMAG-Amine**. In general, the higher the amount of antibody/protein per milligram of **SiMAG-Amine**, the higher will be the degree of magnetic particle surface coating with the protein.

Protocol:

The following protocol describes the coupling of biomolecules on 10 mg particles. The procedure can be scaled up by adjusting volumes of required reagents.

1. Wash the **SiMAG-Amine** particles 2 x with 1 ml MES buffer using the magnetic separator.
2. After the second wash step resuspend the magnetic particles in 0.25 ml MES buffer. Add 0.2 ml Formaldehyde and mix by vortex.
3. Add ligands (e.g. 50 µg protein dissolved in ddH_2O) to the suspension and incubate by a shaker for two hours at room temperature.
4. Wash the particles 3 x with 1 ml PBS.
5. Resuspend the particles in Blocking/Storage buffer.