Enzymatic Synthesis

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Biocatalytic Friedel–Crafts Alkylation Using Non-natural Cofactors**

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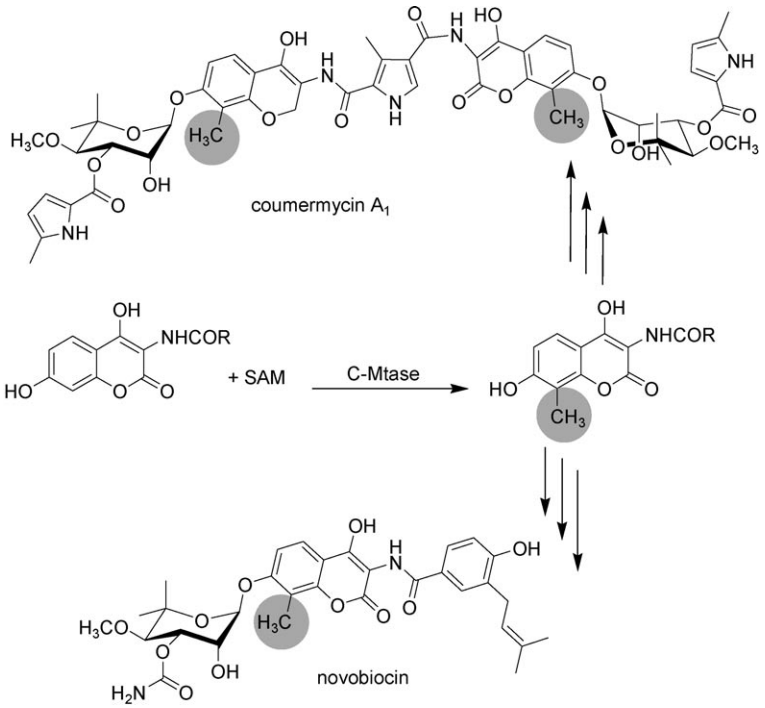
The formation of C–C bonds is a central aspect of synthetic organic chemistry. However, in biocatalysis only few enzymes capable to perform this reaction are known, among which aldolases, transketolases, and hydroxynitril lyases have been investigated thoroughly.[1] Some have even found their way into industrial applications.[2]

Friedel–Crafts alkylation is a classic organic reaction of great importance. However, in particular for large scale application, this transformation is ecologically very critical and regiospecific monoalkylation is difficult to achieve. Therefore, an environmentally friendly and selective alternative would be highly desirable.

In nature methyl groups are selectively introduced into reactive aromatic rings by methyltransferases (Mtases), in particular with *S*-adenosyl-L-methionine (SAM) as the cofactor. Furthermore, enzyme-catalyzed reactions are important for access to isoprenoids. Also, prenylation of aromatic rings has been performed.[3] For phenylalanine ammonia lyases a Friedel–Crafts-type mechanism has been proposed.[4]

Recently, it has been shown that besides the methyl group other alkyl, alkenyl, and alkinyl groups can be introduced into *S*-adenosyl-L-homocystein. These modified cofactors of transferases were used for a sequence-specific alkylation of DNA.[5]



Scheme 1. C-Mtases involved in the biosynthesis of the antibiotics coumermycin A_1 in *Streptomyces rishiriensis* and Novobiocin in *Streptomyces spheroides*.

Having cofactor and modified cofactors in hand, we investigated the possibility of alkylation of aromatic substrates, thus transferring the biosynthesis into the laboratory (Scheme 1).

Aminocoumarins are antibiotics produced by some *Streptomyces* species and are targets for the methyl transfer from the natural cofactor SAM. The Mtase A and B are involved in the biosynthesis[6] of the antibiotics coumermycin A_1[7] (produced by *Streptomyces rishiriensis*) and novobiocin[8] (produced by *Streptomyces spheroides*; Scheme 1).

SAM analogues were synthesized by modifying the strategy published by Klimašauskas, Weinhold, and co-workers.[9] *S*-Adenosyl-L-homocysteine (SAH) was alkylated by seven different alkyl bromides using formic acid as the solvent and AgOTf as a Lewis acid activator and catalyst. We observed quantitative conversion in less than 2 days (average reaction time 24 h; Table 1). The chemical synthesis of SAM analogues results in approximately 1:1 diastereomeric mixtures at the sulfonium center. In the first screenings the diastereomers were separated by preparative HPLC and used as cofactors for the alkylation of coumarin compound **3a**. Both epimers were accepted by the enzymes NovO and CouO

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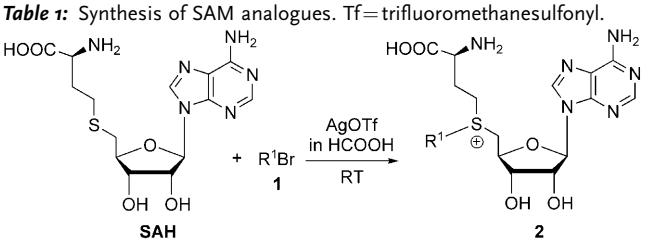
(Mtase A and B) with only slight difference in the conversion after 24 hours. Therefore, the tedious epimer separation was not undertaken. For all following experiments the crude diastereomeric mixture of SAM analogues were performed without further purification and separation of the diastereomers.

The methyltransferase gene *novO* found in *Streptomyces spheroides*[10] shows higher sequence homology to *couO*, which is the methyltransferase gene in *Streptomyces rishiriensis*[11] (84% identity on protein level). These Mtases were heterologously expressed in *E. coli*. Both enzymes were purified as Strep-tagged proteins by affinity chromatography. NovO shows a K_M value of 26.7 μM for substrate **3b** and the K_M value of CouO for substrate **3a** is 64.4 μM. The activity assay is based on HPLC/MS analysis of the enzymatic transformation. For the activity screening model substrates with high similarity to the natural substrates of these enzymes were chosen and synthesized (e.g. **3a** and **3b**; see the Supporting Information).

The biocatalytic transformations were performed in small shaked vials on a 1 mL scale (1 mM substrate) using the crude lysate of the expression in *E. coli* in the presence of 10% DMSO (dimethyl sulfoxide) at 35°C for 24 hours (see the Supporting Information). Some of the reactions were performed on a larger scale (30 mg substrate, 3 mM solution) and the products were isolated. At first coumarin derivatives were chosen as substrates and the results are given in Table 2.

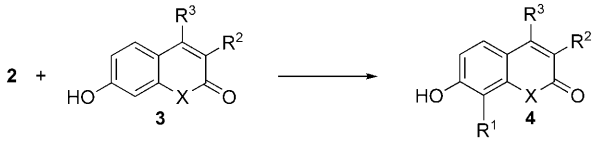
The methylation of coumarin **3a** is not surprising owing to the high structural similarity to the natural substrate. However, besides methyl other alkyl groups can be introduced

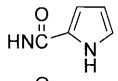
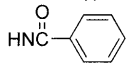
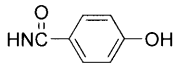
Table 1: Synthesis of SAM analogues. Tf = trifluoromethanesulfonyl.



Alkyl halide	R^1	Product
1a	CH_3	**2a**
1b	$CH_2{=}CHCH_2$	**2b**
1c	$CH_3CH{=}CHCH_2$	**2c**
1d	$CH{\equiv}CCH_2$	**2d**
1e	$CH_3C{\equiv}CCH_2$	**2e**
1f	$C_6H_5CH_2$	**2f**

Table 2: Enzymatic synthesis of alkyl coumarin derivatives.

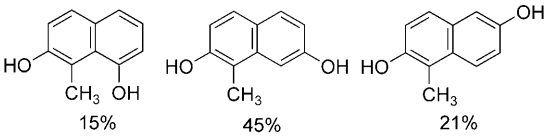


		Substrate		Product
	X	R^2	R^3	$R^1{=}CH_3$
3a	O		OH	**4a**
3b	O		OH	**4b**
3c	O		OH	**4c**
3d	O		OH	**4d**
3e	O	CH_2COOH	CH_3	**4e**
3f	NH	H	CH_3	**4f**

Substr.	Product	NovO[a]	CouO[a]	Substr.	Product	NovO[a]	CouO[a]	Substr.	Product	NovO[a]	CouO[a]
2a **3a**	**4a**: $R^1 = CH_3$	>99	>99	**2a** **3b**	**4b**: $R^1 = CH_3$	>99	96	SAM **3c**	**4c**: $R^1 = CH_3$	>99	>99
2b **3a**	**4a**: $R^1 = CH_2{=}CHCH_2$	>99	>99	**2b** **3b**	**4b**: $R^1 = CH_2{=}CHCH_2$	96	42	SAM **3d**	**4d**: $R^1 = CH_3$	>99	>99
2c **3a**	**4a**: $R^1 = CH_3CH{=}CHCH_2$	42	38	**2c** **3b**	**4b**: $R^1 = CH_3CH{=}CHCH_2$	30	11	SAM **3e**	**4e**: $R^1 = CH_3$	–	30
2d **3a**	**4a**: $R^1 = CH{\equiv}CCH_2$	99	99	**2d** **3b**	**4b**: $R^1 = CH{\equiv}CCH_2$	35	11	SAM **3f**	**4f**: $R^1 = CH_3$	–	10
2e **3a**	**4a**: $R^1 = CH_3C{\equiv}CCH_2$	41	77	**2e** **3b**	**4b**: $R^1 = CH_3C{\equiv}CCH_2$	28	–				
2f **3a**	**4a**: $R^1 = C_6H_5CH_2$	40	45	**2f** **3b**	**4b**: $R^1 = C_6H_5CH_2$	24	21				

[a] % conversion was determined by HPLC analysis.

regiospecifically into C8 of the 4,7-dihydroxycoumarin system. Furthermore, other substituent patterns at C3 are possible. These transformations are interesting because they are the first examples of biocatalytic Friedel–Crafts alkylations. It was therefore very gratifying that also other substrates were accepted by CouO as shown in Scheme 2.



Scheme 2. Methylation products of naphthalenediols catalyzed by CouO (% conversion after 24 h).

There seem, however, to be quite stringent structure requirements since naphthalenediols with a 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, or 2,3-substitution pattern gave no reaction. In addition, resorcinol and phloroglucinol were not accepted.

In summary a biocatalytic equivalent for the Friedel–Crafts alkylation is described. The enzymes are SAM dependent methyltransferases which are capable of accepting modified cofactors and yield not only methylated but also allyl, propargyl, and benzylated arenes in moderate to high yields with excellent regioselectivity. Only monosubstituted products were formed, even if a large excess of SAM and analogues was applied. Gratifyingly, the substrate acceptance of the Mtases is broader than expected. Naphthalene derivatives can replace the coumarin scaffold. This concept may serve as the beginning of a "green" and selective Friedel–Crafts alkylation.

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