Synthesis of Different β-Alkylated Pimelic Acids

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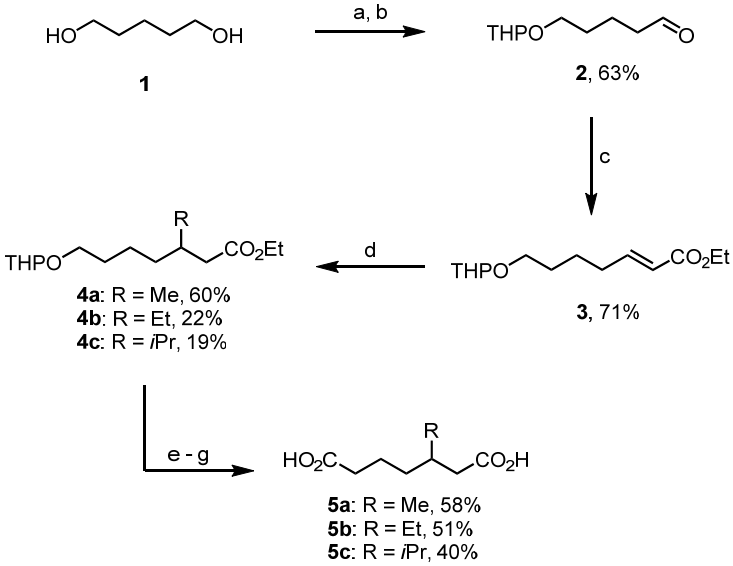
To study the anaerobic microbial processes of metabolic pathways, it is important to synthesize reference compounds to use them as standards for GC-analysis and comparison with the data from biochemical experiments. Therefore, we developed a synthetic route to different β-alkylated pimelic acids as reference for compounds preserved from the bacterial strain *pCyN1*.

Introduction

Within the biological carbon cycle on earth anaerobic microbial processes play the major role in subsurface transformations of organic compounds. In these anoxic environments anaerobic microorganisms have developed special strategies to use alkanes as a source of energy.[1] To learn more about the metabolic processes of the anaerobic microorganisms cultivation experiments can be performed. Furthermore, there is the possibility to access selected compounds by chemical synthesis. These products can be used as standards for GC-analysis and compared with the data from cultivation experiments.

Organic Synthesis

A synthetic route to three racemic β-alkylated pimelic acids **5a-5c** consisting of seven steps starting from 1,5-pentanediol (**1**) was developed. First step was the acid catalyzed protection of one alcohol group with DHP followed by Swern oxidation of the other one and Horner-Wadsworth-Emmons reaction to obtain the Michael acceptor **3**. After copper catalyzed conjugated addition with different Grignard reagents and acidic cleavage of the THP group the pimelic acids **5a-5c** are obtained after oxidation of the alcohol group using TEMPO as catalyst and $PhI(OAc)_2$ as the oxidant and ester saponification (Scheme 1).



1

2, 63%

3, 71%

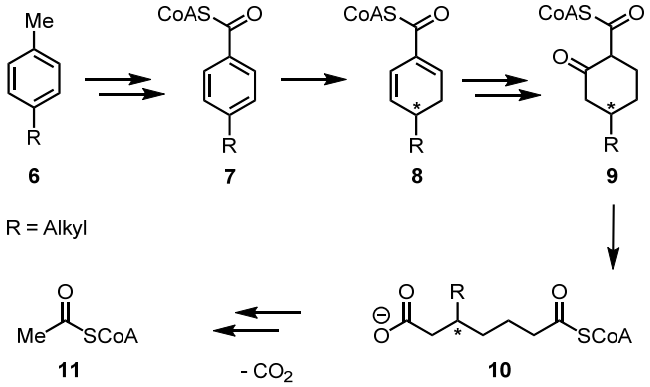
4a: R = Me, 60%
4b: R = Et, 22%
4c: R = *i*Pr, 19%

5a: R = Me, 58%
5b: R = Et, 51%
5c: R = *i*Pr, 40%

Scheme 1: a) DHP, PPTS, DCM, 19 h, 23°C; b) $(COCl)_2$, DMSO, NEt_3, 3 h, -78°C; c) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF, 60 min, 5–23°C; d) RMgX, 5 mol% CuTC, TMSCl, Et_2O, 16 h, -78°C; e) HCl–H_2O, EtOH/DCM 4:1, 3 h, 80°C; f) 20 mol% TEMPO, $PhI(OAc)_2$, MeCN/H_2O 1:1, 16 h, 23°C; g) NaOH–H_2O, MeOH, 25 h, 23°C. DHP = 3,4-dihydro-*2H*-pyran, PPTS = pyridinium *p*-toluenesulfonate, X = Cl or Br, CuTC = copper(I)-thiophene-2-carboxylate, TEMPO = (2,2,6,6-tetramethylpiperidin-1-yl)oxyl.

Microbiological Background

The strain *pCyN1* is able to survive and grow under anoxic conditions just by being fed with 4-isopropyltoluene or similar compounds **6**. A suggestion of the central pathway of the metabolic process leading to the final products acetyl–CoA **11** and CO_2 is given in Scheme 2.[2] After oxidation of the methyl group a "biological Birch reduction" leads to the formation of a stereocenter in compound **8**. This stereoinformation is expected to be retained in the subsequent products which can be detected by harvesting the strain. Especially the β-alkylated pimelic acids **10** seem to be a good system to explore the metabolic mechanism.



Scheme 2: Central degradation pathway of *pCyN1* for alkylated toluene derivatives **6**.[2]

Comparison of Synthetic Compounds and Extract

The comparison of the synthetic racemic β-isopropyl pimelic acid (**5c**) with the extracted compound from *pCyN1* shows, that addition of 4-isopropyltoluene (**6**) as nutrient leads to the formation of only one enantiomer of the acid **5c** (Figure 1). Furthermore, it was shown that by using 4-ethyltoluene (**6**) the ethylated pimelic acid **5b** can be found. This supports that our proposed central metabolic pathway is accurate.

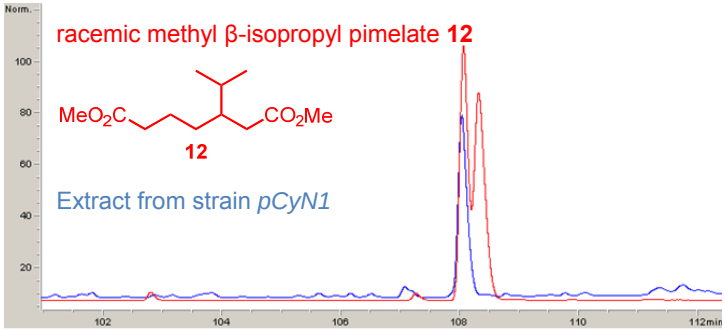


Figure 1: GC-comparison of the synthetic compound **12** (red) with the extract from *pCyN1* (blue). The carboxylic acids were esterified with diazomethane prior to analysis to obtain a better separation of the enantiomers.

[1] R. Jarling, *Transformations of hydrocarbons in anaerobic bacteria*, Dissertation, Universität Oldenburg, **2016**.
[2] A. Strijkstra, K. Trautwein, R. Jarling, L. Wöhlbrand, M. Dörries, R. Reinhardt, M. Drozdowska, B. T. Golding, H. Wilkes, R. Rabus, *Appl. Environ. Microbiol.* **2014**, *80*, 7592–7603.