Optimized Biocatalytic Synthesis of 2-Selenopyrimidine Nucleosides by Transglycosylation**

Katja F. Hellendahl*, Felix Kaspar*, Xinrui Zhou, Zhaoyi Yang, Zhen Huang, Peter Neubauer, and Anke Kurreck*
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**Author Contributions** (with definitions as recommended by Brand et al.[1])

Conceptualization, K.F.H., F.K., P.N. and A.K.; methodology, K.F.H., F.K. and A.K.; software, F.K.; validation, K.F.H.; formal analysis, K.F.H and F.K.; investigation, K.F.H and F.K.; resources, X.Z., Z.Y., Z.H., A.K and P.N.; writing – original draft, K.F.H and F.K; writing – review and editing, K.F.H., F.K., X.Z., Z.Y., Z.H., A.K. and P.N.; visualization, K.F.H, F.K. and A.K., supervision, A.K. and P.N.; project administration K.F.H, F.K., A.K. and P.N.; Funding acquisition, A.K. and P.N.

All authors have read and agree to the published version of the manuscript.

**Data availability**

All data depicted visually in the items in the main text as well as in the Supplementary Information is available from an externally hosted Supporting Information.[2]
Figure S1. Plots for the pKₐ determination of 1 (A) and 2 (B). The 2-Se-nucleobases were dissolved to a concentration of 100 µM in 50 mM MOPS buffer. The pH was adjusted with HCl and NaOH at RT. Samples were analysed by spectral unmixing² using the isosbestic points of 282 nm for 1 and 279 nm for 2. The pKₐ for 1 was 7.21 ± 0.03 and 7.49 ± 0.006 for 2.

Figure S2. Solubility of 20 mM (A) and 10 mM (B) of 1 and 2 in 50 mM MOPS pH 7 and 50 mM Glycine pH 9 at RT.
Figure S3. Specific activity of PyNP Y04 in phosphorolysis reactions (A) under different conditions (B). Standard phosphorolysis reactions were performed with 1 mM a, 50 mM K$_2$HPO$_4$ in 50 mM glycine/NaOH buffer pH 9 in a total volume of 0.5 mL at 80 °C. Final concentrations of PyNP Y04 of 150 to 750 ng mL$^{-1}$ were applied. To study the impact of substrates (b, c), temperature and reducing conditions on the enzyme activity. Reactions were performed at 60°C and pH 7, under N$_2$ atmosphere or with the addition of 5 mM DTT. Samples were analysed via spectral unmixing.\cite{ref11}
**Figure S4.** Specific activity of PyNP Y04 for the synthesis of 2b via direct glycosylation. Reactions were performed with 1 mM 2, 10 mM b’ and 70 µg mL⁻¹ PyNP Y04 in 50 mM glycine/NaOH pH 9 in a total volume of 250 µL at 60 °C. Samples were analysed via spectral unmixing.[2]

**Figure S5.** Stability of 5 mM 2 at 80 °C in 50 mM glycine/NaOH buffer pH 9 without additives (A), with 5 mM DTT (B), saturated with nitrogen (C) and the combination of 5 mM DTT and nitrogen (D). Samples were diluted to 1 mM in MeOH and analysed at 260 nm (black) and 307 nm (red) by HPLC. Retention time of 2 is 6 min and the degradation peak is at 12.44 min.
Table S1 Overview of equilibrium constants and prices of potential sugar donors.

| Sugar donor       | Equilibrium constant | Price [€ g⁻¹][b]          |
|-------------------|-----------------------|---------------------------|
|                   |                       |                           |
| Uridine           | 0.18[a]               | 4.5                       |
|                   |                       | (100 g 450 €)             |
| Ribose            | 5-Ethynyluridine      | 11,000                    |
|                   | 0.61[a]               | (5 mg 55 €)               |
|                   | 7-Methylguanosine     | 1188                      |
|                   |                       | (250 mg 297 €)            |
| Deoxyribose       | Thymidine             | 13.52                     |
|                   | 0.15[a]               | (25 g 338 €)              |
|                   | 5-Ethynyl-2'-deoxyuridine | 1948                |
|                   | 0.35[a]               | (500 mg 974 €)            |

[a] at 40 °C from [3–5]
[b] Prices were calculated from the biggest pack size available for Germany on the Sigma-Aldrich website. Last access 28.09.2020.

Table S2 Purification of 2-Se-nucleosides by semi-preparative HPLC.

| Product | Total sample volume [mL] | HPLC gradient                                      |
|---------|--------------------------|----------------------------------------------------|
| 1a      | 40                       | Initial: 3% ACN, 97% water                         |
|         |                          | 10 min: 3% ACN, 97% water                          |
|         |                          | 18 min: 40% ACN, 60% water                         |
| 1b      | 30                       | 18.5 min: 3% ACN, 97% water                        |
|         |                          | 20 min: 3% ACN, 97% water                          |
| 2a      | 40                       | Initial: 3% ACN, 97% water                         |
|         |                          | 7 min: 3% ACN, 97% water                           |
|         |                          | 18 min: 40% ACN, 60% water                         |
| 2b      | 10                       | 18.5 min: 3% ACN, 97% water                        |
|         |                          | 20 min: 3% ACN, 97% water                          |

A flow rate of 21.24 mL min⁻¹ was used. Acetonitrile (ACN) and deionized water were applied as solvents. Samples were analysed at 210 nm.
Figure S6. Product 1a was analysed by HPLC (A) and ESI-Orbitrap-MS (B: extracted ion chromatogram, C: MS spectrum).
Figure S7. Product 1b was analysed by HPLC (A) and ESI-Orbitrap-MS (B: extracted ion chromatogram, C: MS spectrum).
Figure S8. Product 2a was analysed by HPLC (A) and ESI-Orbitrap-MS (B: extracted ion chromatogram, C: MS spectrum).
Figure S9. Product 2b was analysed by HPLC (A) and ESI-Orbitrap-MS (B: extracted ion chromatogram, C: MS spectrum).
References

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