Regioselectivity of Cobalamin-Dependent Methyltransferase Can Be Tuned by Reaction Conditions and Substrate

Simona Pompei, Christopher Grimm, Judith E. Farnberger, Lukas Schober, and Wolfgang Kroutil*
Figure S1. Regioselectivity in buffer for substrates 1b-f.
Table S1. Co-solvent screening (10% v/v). [a] Conversion (conv.%) of acceptor into products after 24 hours based on the limiting reagent. *Conversion based on catechol formation. [b] The regioisomer in excess (indicated as m-2n) was calculated after 24 hours as the percentage of the one isomer out of the total of products formed (considered as 100%). Positive values indicate the m-2n to be in access, whereas negative values denote p-2n to be in access. The error was between 0.6 and 1% (average ±0.8%).

| Buffer | DMSO | MeOH | THF | Dioxane | EtOH | Acetone | THT |
|--------|------|------|-----|---------|------|---------|-----|
| Substr | conv.[a] | 2n[b] | conv | 2n | conv | 2n | conv | 2n | conv | 2n | conv | 2n | conv | 2n | conv | 2n |
| 1b     | 60%  | 71%  | 60% | 60% | 70% | 75% | 6%  | 100%| 21% | 72% | 52% | 83% | 65% | 77% | 50% | 77% |
| 1c     | 91%  | 70%  | 93% | 54% | 83% | 54% | 87% | 100%| 85% | 83% | 89% | 73% | 95% | 63% | 77% | 65% |
| 1d     | 66%  | 69%  | 71% | 74% | 70% | 76% | 2%  | 100%| 41% | 72% | 62% | 83% | 79% | 73% | 21% | 90% |
| 1e     | 41%  | 61%  | 34% | 59% | 28% | 76% | n.d.| n.d.| 6%  | 54% | 12% | 62% | 22% | 68% | 10% | 61% |
| 1f     | 52%  | 48%  | 80% | 61% | 79% | 61% | 70% | 70% | 64% | 78% | 74% | 70% | 79% | 64% | 40% | 61% |
| 1g     | 71%  | 50%  | 68% | -55%| 79% | 55% | 2%  | 76% | 27% | 58% | 58% | 63% | 70% | 60% | 75% | 61% |
| 1h     | 75%  | -55% | 81% | -56%| 75% | 55% | 9%  | 68% | 19% | 59% | 39% | 50% | 68% | 50% | 78% | 52% |
| 1i     | 38%  | -66% | 83% | -62%| 75% | -53%| 2%  | -77%| 11% | -75%| 12% | -66%| 22% | -70%| 43% | -70%|

Table S2. Co-solvent screening (5-15% v/v). [a] Conversion (conv.%) of acceptor into products after 24 hours based on the limiting reagent. *Conversion based on catechol formation. [b] The regioisomer in excess (indicated as m-2) was calculated after 24 hours as the percentage of the one isomer out of the total of products formed (considered as 100%). The error was between 0.6 and 1% (average ±0.8%).

| Buffer | THF 5% v/v | THF 15% v/v | EtOH 15% v/v | MeOH 15% v/v |
|--------|------------|-------------|--------------|--------------|
| Substr | conv.[a]   | 2n[b]       | conv.[a]     | 2n[b]        | conv.[a]     | 2n[b]        | conv.[a]     | 2n[b]        |
| 1b     | 30%        | 73%         | n.d.         | n.d.         | 20%          | 68%          | -            | -            |
| 1c     | 90%        | 81%         | 25%          | 98%          | -            | -            | -            | -            |
| 1d     | 68%        | 76%         | n.d.         | n.d.         | 14%          | 100%         | -            | -            |
| 1e     | n.d.       | n.d.        | n.d.         | n.d.         | -            | 21%          | 6000         |               |
Figure S2. Regioselectivity for substrates 1b-e with different co-solvents concentrations (5-15% v/v).

| pH  | m-2d [b] [%] | conv. [a] [%] |
|-----|--------------|---------------|
| 7.5 | 92           | 8             |
| 9   | 94           | 6             |

Table S3. Additivity of pH and co-solvent (EtOH) effect. [a] Conversion (conv. [%]) of acceptor 1d into products (m-2d and p-2d) after 24 hours based on the limiting reagent. [b] Vanillin (indicated as m-2d) was calculated after 24 hours as the percentage of the one isomer out of the total of products formed (considered as 100%).

| pH  | conv. [a] [%] | m-2d [b] [%] | conv. [%]  | p-2i [%] |
|-----|---------------|--------------|------------|----------|
| 6.5 | 67            | 79           | 77         | 68       |
| 7.0 | 70            | 81           | 45         | 71       |
| 7.5 | 66            | 84           | 50         | 71       |
| 8.0 | 66            | 83           | 46         | 72       |
| 9.0 | 60            | 83           | 43         | 72       |
| 10.0| 56            | 83           | 28         | 71       |

Table S4. Effect of the pH. [a] Conversion (conv. [%]) of acceptor 1d into products (m-2d and p-2d) after 24 hours based on the limiting reagent. [b] Isomer (indicated as m-2d or p-2i) was calculated after 24 hours as the percentage of the one isomer out of the total of products formed (considered as 100%). The error was between 0.6 and 1% (average ±0.8%).
### Table S5. LogP of water miscible co-solvents used and corresponding regioselectivity and normalized regioselectivity (% m-2 formed over % m-2 in DMSO). Positive values indicate the m-2n, whereas negative values denote p-2n. The error was between 0.6 and 1% (average ±0.8%).

| Co-solvent | logP | m-2b [%] | m-2b - normalized | m-2c [%] | m-2c - normalized | m-2d [%] | m-2d - normalized |
|------------|------|----------|--------------------|----------|--------------------|----------|--------------------|
| DMSO       | -1.35| 60       | 1                  | 54       | 1                  | 74       | 1                  |
| methanol   | -0.69| 75       | 1.25               | 54       | 1                  | 76       | 1.027027           |
| dioxane    | -0.27| 72       | 1.2                | 72       | 1.333333           | 72       | 0.972973           |
| acetone    | -0.24| 77       | 1.283333           | 68       | 1.259259           | 73       | 0.986486           |
| ethanol    | -0.18| 83       | 1.383333           | 73       | 1.351852           | 83       | 1.121622           |
| THF        | 0.46 | 100      | 1.666667           | 100      | 1.851852           | 100      | 1.351351           |

| Co-solvent | logP | m-2e [%] | m-2f [%] | m-p-2g [%] | m-p-2h [%] | p-2i [%] |
|------------|------|----------|----------|------------|------------|----------|
| DMSO       | -1.35| 59       | 59       | -55        | -56        | -62      |
| methanol   | -0.69| 76       | 61       | 55         | 55         | -53      |
| dioxane    | -0.27| 54       | 78       | 58         | 59         | -75      |
| acetone    | -0.24| 68       | 64       | 60         | 50         | -70      |
| ethanol    | -0.18| 62       | 70       | 63         | 50         | -66      |
| THF        | 0.46 | -        | 70       | 76         | 68         | -77      |

For Hydroxytyrosol 1f: (3,4-Dihydroxyphenyl)acetic methyl ester 5f.

2-(2,2-Dimethylbenzo[1,3]dioxol-5-yl)acetic methyl ester 4f.

2-(2,2-Dimethylbenzo[1,3]dioxol-5-yl)ethanol 2f.
Reference material: 5-(2-hydroxyethyl)-2-methoxyphenol $p$-$2f$.

Reference material: 5-(1-hydroxyethyl)-2-methoxyphenol $p$-$3h$.

5-ethyl-2-methoxyphenol $p$-$2h$.

Scheme S1. Synthesis of reference and starting material (for procedures see main paper)
Figure S3. (3,4-Dihydroxyphenyl)acetic methyl ester 5f \(^1\)H NMR.

\(^1\)H NMR (300 MHz, MeOD) \(\delta \) 6.70 – 6.68 (m, 2H), 6.58 – 6.52 (m, 1H), 3.66 (s, 3H), 3.46 (s, 2H).
Figure S4. 3,4-Dihydroxyphenylacetic methyl ester 5f $^{13}$C NMR.

$^{13}$C NMR (75 MHz, MeOD) $\delta$ 174.53, 146.27, 145.42, 126.89, 121.60, 117.30, 116.26, 52.36, 41.15.
Figure S5. 2-(2,2-Dimethylbenzo[1,3]dioxol-5-yl)acetic methyl ester 4f $^1$H NMR.

$^1$H NMR (300 MHz, MeOD) δ 6.65 (d, $J = 5.3$ Hz, 3H), 3.66 (s, 3H), 3.52 (s, 2H), 1.63 (s, 6H).
Figure S6. 2-(2,2-Dimethylbenzo[1,3]dioxol-5-yl)acetic methyl ester 4f $^{13}$C NMR.

$^{13}$C NMR (75 MHz, MeOD) $\delta$ 174.17, 148.91, 147.89, 128.51, 122.90, 119.02, 110.40, 108.86, 52.42, 41.32, 25.91.
Figure S7. 2-(2,2-Dimethylbenzo[1,3]dioxol-5-yl)ethanol 3f $^1$H NMR.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 6.69 – 6.56 (m, 3H), 3.79 (t, 2H), 2.75 (t, 2H), 1.64 (s, 6H).
**Figure S8.** 2-(2,2-Dimethylbenzo[1,3]dioxol-5-yl)ethanol 3f $^{13}$C NMR.

$^{13}$C NMR (75 MHz, CDCl$_3$) δ 147.83, 146.25, 131.55, 121.42, 117.90, 109.25, 108.29, 63.95, 39.07, 26.02.
Figure S9. Hydroxytyrosol 1f $^1$H NMR.

$^1$H NMR (300 MHz, MeOD) $\delta$ 6.66 (m, $J = 7.3$, 5.0 Hz, 1H), 6.52 (m, $J = 8.0$, 2.1 Hz, 2H), 3.67 (t, $J = 7.3$ Hz, 2H), 2.66 (t, $J = 7.2$ Hz, 2H).
Figure S10. Hydroxytyrosol 1f $^{13}$C NMR.
$^{13}$C NMR (75 MHz, MeOD) $\delta$ 146.14, 144.62, 131.77, 121.19, 117.05, 116.29, 64.60, 39.67.
Figure S11. 5-(2-hydroxyethyl)-2-methoxyphenol $p$-2f $^1$H NMR.
$^1$H NMR (300 MHz, MeOD) $\delta$ 6.90 – 6.81 (m, 1H), 6.76 – 6.63 (m, 2H), 3.84 (s, 3H), 3.73 (t, $J = 7.2$ Hz, 2H), 2.73 (t, $J = 7.2$ Hz, 2H).
Figure S12. 5-(2-hydroxyethyl)-2-methoxyphenol $\rho$-2f $^{13}$C NMR.

$^{13}$C NMR (75 MHz, MeOD) $\delta$ 147.50, 147.40, 133.19, 121.09, 116.99, 112.84, 64.46, 56.47, 39.63.
Figure S13. 5-(1-hydroxyethyl)-2-methoxyphenol p-3h $^1$H NMR.

$^1$H NMR (300 MHz, MeOD) δ 6.85 (dd, $J = 5.3$, 2.9 Hz, 2H), 6.78 (dd, $J = 8.3$, 2.0 Hz, 1H), 4.70 (q, $J = 6.5$ Hz, 1H), 3.82 (s, 3H), 1.39 (d, $J = 6.5$ Hz, 3H).
Figure S14. 5-(1-hydroxyethyl)-2-methoxyphenol \( \rho \text{-}3h \) \( ^{13} \text{C} \) NMR.

\( ^{13} \text{C} \) NMR (75 MHz, MeOD) \( \delta \) 148.10, 147.36, 140.49, 117.81, 113.67, 112.46, 70.53, 56.43, 25.46.
Figure S15. 5-ethyl-2-methoxyphenol p-2h $^1$H NMR.

$^1$H NMR (300 MHz, MeOD) $\delta$ 6.77 (d, $J$ = 8.1 Hz, 1H), 6.69 – 6.52 (m, 2H), 3.78 (s, 3H), 2.48 (q, $J$ = 7.6 Hz, 2H), 1.15 (t, $J$ = 7.6 Hz, 3H).
Figure S16. 5-ethyl-2-methoxyphenol $p$-2h $^{13}$C NMR.

$^{13}$C NMR (75 MHz, MeOD) $\delta$ 147.32, 147.00, 138.55, 119.78, 115.89, 112.85, 56.49, 29.19, 16.36.
Figure S17. Example of HPLC chromatograms. Commercially available references.
Figure S18. Example of HPLC chromatograms of biotransformations. Top chromatogram: biotransformation in the presence of 10% v/v of MeOH analyzed after 24 h; a) catechol, b) substrate 1b, c) product m-2b, d) product p-2b, e) guaiacol; Bottom chromatogram: biotransformation in the presence of 10% v/v of THF analyzed after 24 h; a) catechol, b) substrate 1b, c) product m-2b, e) guaiacol
Figure S19. Example of HPLC chromatograms of biotransformations with XXXYX.
Figure S20. (A) HPLC chromatogram of biotransformation and (B) spiked chromatogram with the commercial available reference isomer 4-(tert-butyl)-2-methoxyphenol.

Figure S21. 1H NMR of 24 mL scale methylation of 4-tert-butylcatechol. The extracted mixture from the biotransformation is reported in red. The three singlet reported at 3.82, 3.80 and 3.78 ppm belong respectively to 4-(tert-butyl)-2-methoxyphenol, guaiacol and 5-(tert-butyl)-2-methoxyphenol, according to the reference material NMRs (reported in the different colors).
**Figure S22.** Example of HPLC calibration curve.

| Compound                | RT [min] method A | RT [min] method B | RT [min] method C | RT [min] method D |
|-------------------------|-------------------|-------------------|-------------------|-------------------|
| Guaiacol                | 17.7              | 20.0              | 14.2              | 21.9              |
| Catechol                | 13.3              | 15.0              | 11.6              | 14.1              |
| Vanillic acid           | 14.2              | 17.0              | n.d.              | n.d.              |
| Isovanillic acid        | 14.5              | 14.4              | n.d.              | n.d.              |
| 3,4-dihydroxybenzoic acid | 11.8              | 13.5              | n.d.              | n.d.              |
| Vanillin                | 15.9              | 18.2              | n.d.              | n.d.              |
| Isovanillin             | 15.7              | 17.9              | n.d.              | n.d.              |
| 3,4-dihydroxybenzaldehyde | 13.2              | 14.4              | n.d.              | n.d.              |
| Ferulic acid            | 16.6              | 19.6              | n.d.              | n.d.              |
| Isoferulic acid         | 16.8              | 20.1              | n.d.              | n.d.              |
| Caffeic acid            | 14.3              | 16.3              | n.d.              | n.d.              |
| Vanillyl alcohol        | 11.8              | n.d.              | n.d.              | n.d.              |
| Substance                                           | Retention Time | Method 1 | Method 2 | Method 3 |
|-----------------------------------------------------|----------------|----------|----------|----------|
| Isovanillyl alcohol                                 | 12.4           | n.d.     | n.d.     | n.d.     |
| 3,4-dihydroxybenzyl alcohol                         | 9.6            | n.d.     | n.d.     | n.d.     |
| 3-Hydroxytyrosol                                    | 10.9           | n.d.     | n.d.     | n.d.     |
| 5-(2-hydroxyethyl)-2-methoxyphenol                  | 13.9           | n.d.     | n.d.     | n.d.     |
| Homovanillyl alcohol                                | 12.6           | n.d.     | n.d.     | n.d.     |
| 4-methylcatechol                                    | 15.9           | n.d.     | n.d.     | n.d.     |
| 2-methoxy-5-methylphenol                            | 19.6           | n.d.     | n.d.     | n.d.     |
| 2-methoxy-4-methylphenol                            | 19.7           | n.d.     | n.d.     | n.d.     |
| 4-ethylcatechol                                     | 18.7           | 21.9     | 14.4     | n.d.     |
| 2-methoxy-5-ethylphenol                             | 20.7           | n.d.     | 16.6     | n.d.     |
| 2-methoxy-4-ethylphenol                             | 20.8           | n.d.     | 16.5     | n.d.     |
| 4-tbutylcatechol                                    | 20.5           | 22.9     | n.d.     | 29.6     |
| 2-methoxy-5-tbutylphenol                            | n.d.           | n.d.     | n.d.     | 35.1     |
| 2-methoxy-4-tbutylphenol                            | n.d.           | n.d.     | n.d.     | 34.9     |

**Table S4.** HPLC retention times and used methods for the screened substrates and products.

**References**

[1] A. Gambacorta, D. Tofani, R. Bernini, A. Migliorini, *J. Agr. Food. Chem.* **2007**, *55*, 3386-3391.