Quantitative and stereo-controlled cross-photocycloaddition of the anticancer drug 5-fluorouracil in a cocrystal

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1) Reagents and Experimental

5-fluorouracil, 1,2-bis(2-pyridyl)ethylene, and dichloromethane were purchased from Sigma-Aldrich. Methanol, isopropanol, and hydrochloric acid were purchased from Fisher Scientific. All chemicals were used as received and without further purification.

Solid-state photocycloadditions were performed as follows: Single crystal samples of 1 were manually ground to a fine powder using a mortar and pestle. The powder was placed between two Pyrex glass plates and irradiated under a medium pressure Hg vapor lamp. The plates were regularly turned to ensure uniform irradiation. Progress of the reaction was monitored via $^1$H NMR spectroscopy.
2) Single crystal X-ray diffraction measurements

Single crystal XRD data were collected on a Bruker Nonius Apex II Kappa CCD single-crystal X-ray diffractometer using MoK$_\alpha$ radiation ($\lambda = 0.71073$ Å). Reflections were harvested from $\phi$ and $\omega$ scans with an intensity threshold of $I > 2\sigma(I)$. Data collection, reduction, and cell refinement were accomplished using the Bruker apexII software suite. Using Olex2,$^1$ structure solution and refinement were accomplished using SHELXT$^2$ and SHELXL$^3$, respectively. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms associated with carbon atoms were refined in geometrically constrained positions.

**Table S1:** Crystal data and structure refinement for 2(5-FU)(2,2’-bpe).

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<thead>
<tr>
<th>Property</th>
<th>Value</th>
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<tr>
<td>Empirical formula</td>
<td>C$<em>{20}$H$</em>{16}$F$_2$N$_6$O$_4$</td>
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<td>Formula weight</td>
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<td>Space group</td>
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<td>$b$/Å</td>
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<tr>
<td>$c$/Å</td>
<td>10.409(5)</td>
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<td>107.757(5)</td>
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<tr>
<td>$\beta$/°</td>
<td>97.878(5)</td>
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<tr>
<td>$\gamma$/°</td>
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<tr>
<td>$\rho$$_{\text{calc}}$/g/cm$^3$</td>
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<tr>
<td>$\mu$/mm$^{-1}$</td>
<td>0.124</td>
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<tr>
<td>F(000)</td>
<td>228.0</td>
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<tr>
<td>Crystal size/mm$^3$</td>
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<tr>
<td>Radiation</td>
<td>MoK$_\alpha$ ($\lambda = 0.71073$ Å)</td>
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<td>$2\theta$ range for data collection/°</td>
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<td>Index ranges</td>
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<tr>
<td>Independent reflections</td>
<td>1732 [R$<em>{int} = 0.0345$, R$</em>{sigma}$ = 0.0411]</td>
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<tr>
<td>Data/restraints/parameters</td>
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<tr>
<td>Goodness-of-fit on F$^2$</td>
<td>1.015</td>
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<tr>
<td>Final R indexes [I&gt;2σ(I)]</td>
<td>$R_1 = 0.0441$, wR$_2 = 0.0982$</td>
</tr>
</tbody>
</table>
Final R indexes [all data]  \( R_1 = 0.0708, wR_2 = 0.1109 \)
Largest diff. peak/hole / e Å\(^{-3}\)  0.29/-0.27
The photoproduct \textbf{fpbcu} was refined as a two-component, non-merohedral twin. Refinement of \textbf{fbpcu} was performed as above with absorption correction for twinned components accomplished using Bruker TWINABS software. The twin law was found to be a two-fold rotation about the (011) axis with final basf = 0.3089(19).

\textbf{Table S2}: Crystal data and structure refinement for \textbf{fbpcu}.

\begin{tabular}{ll}
Empirical formula & C_{16}H_{13}N_{4}O_{2}F \\
Formula weight & 312.30 \\
Temperature/K & 296.15 \\
Crystal system & triclinic \\
Space group & P\bar{1} \\
a/Å & 7.8358(8) \\
b/Å & 12.4452(12) \\
c/Å & 15.6290(15) \\
α/° & 98.513(5) \\
β/° & 102.020(5) \\
γ/° & 101.749(5) \\
Volume/Å³ & 1430.3(2) \\
Z & 4 \\
ρ_{calc} g/cm³ & 1.450 \\
μ/mm⁻¹ & 0.108 \\
F(000) & 648.0 \\
Crystal size/mm³ & 0.27 × 0.12 × 0.06 \\
Radiation & MoKα (λ = 0.71073) \\
2Θ range for data collection/° & 5.438 to 50.852 \\
Index ranges & -9 ≤ h ≤ 9, -15 ≤ k ≤ 14, 0 ≤ l ≤ 18 \\
Reflections collected & 15496 \\
Independent reflections & 5257 [R_{int} = 0.0341, R_{sigma} = 0.0596] \\
Data/restraints/parameters & 5257/0/432 \\
Goodness-of-fit on F² & 1.017 \\
Final R indexes [I≥2σ (I)] & R₁ = 0.0486, wR₂ = 0.0991 \\
Final R indexes [all data] & R₁ = 0.0945, wR₂ = 0.1175 \\
Largest diff. peak/hole / e Å⁻³ & 0.22/-0.21
\end{tabular}
3) Powder X-ray diffraction measurements

Powder X-ray diffraction data were collected from samples mounted on glass slides by a Siemens D5000 X-ray diffractometer using CuK$_{\alpha1}$ radiation ($\lambda = 1.54056$ Å) (scan type: locked coupled; scan mode: continuous; step size: 0.02°).

**Figure S1.** Overlay of experimental X-ray powder patterns of 2(5-FU)(2,2’-bpe) before reaction (bottom, red) and after reaction (top, blue).
4) NMR spectra and analysis.

$^1$H NMR spectra for 2(5-FU)(2,2′-bpe) were collected using a Bruker Avance-400 NMR spectrometer operating at 400 MHz. $^1$H chemical shifts were referenced from the $^1$H chemical shifts of DMSO-D$_6$ (2.50 ppm).

The final photoproduct fbpcu was characterized using Bruker Avance-400 and Avance-600 NMR spectrometers operating at 400 and 600 MHz, respectively. $^1$H and $^{13}$C chemical shifts were referenced from the $^1$H and $^{13}$C chemical shifts of DMSO-D$_6$ ($^1$H, 2.50 ppm; $^{13}$C, 39.5 pm). A battery of one- and two-dimensional (1D and 2D) homonuclear and $^1$H-$^{13}$C heteronuclear experiments [$^1$H, $^{13}$C, 2D correlated spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC)] were performed on the final product to determine resonance assignments. Gradient-assisted versions of the pulse sequences and inverse detection were used for these 2D experiments. $^1$H spectral widths and 90° pulse widths are optimized for each sample. A recycle delay (D1) of 4.0s was used in all the 2D experiments. Typical parameters for the NMR experiments were as follows: $^1$H [time domain data points (TD), 32k; NS, 32], $^{13}$C (TD, 64k; NS, 10k), 2D COSY (TD, 2k; TD1, 150; NS, 16; DS, 32), $^{13}$C-$^1$H HSQC (TD, 2k; TD1, 256; NS, 16; DS, 128) and $^{13}$C-$^1$H HMBC (TD, 4k; TD1, 256; NS, 32; DS, 64). TD, NS, and DS refer to time domain data points, number of scans, and dummy scans, respectively. All NMR data were processed with the TOPSPIN 1.3 suite of software programs. The 1D $^1$H data were processed with zero-filling to 64k data points and 0.2 Hz exponential line broadening, whereas $^{13}$C spectra were processed with zero-filling to 128k data points and 1.0 Hz of exponential line broadening. The 2D NMR data were processed with the zero-filling to 2,048 points and 1,024 points in acquisition and second dimension, respectively.
The $^1$H spectrum with resonance assignments for $2(5$-FU)$2,2'$-bpe is provided below (Figures S2 and S3). Resonances for 5-FU ($H_a$, $H_b$, and $H_c$) were assigned according to published spectra. Resonances for $2,2'$-bpe were assigned according to approximate chemical shifts and coupling constants. The signal at 7.70 ppm was ascribed to the olefinic proton $H_h$ based on the singlet multiplicity, relative integrated intensity equivalence of two protons, and the observed disappearance of the signal through the course of the photoreaction. Assignment of the pyridyl resonances was supported by coupling constants. The largest coupling constant observed in the signals at 8.61 and 7.30 ppm ($^3J = 4.75$ Hz) is indicative of the ortho-aromatic relation of $H_d$ and $H_e$. Similarly, the strongest coupling observed in the signals at 7.81 and 7.63 ppm ($^3J = 7.7$ Hz) likewise supports the ortho relation of $H_f$ and $H_g$. Note that the protons with a single ortho proton partner ($H_d$ and $H_g$) are expected to show splitting patterns with one large (for ortho) and two small coupling constants (for meta and para).
Figure S5.

Figure S2.

H NMR of 2(5-FU) (before reaction).

Figure S3.

Expansion of H NMR of 2(5-FU) (before reaction).

Figure S4.

H NMR of 2(5-FU) (after reaction).
$^1$H spectrum of (fbpcu) with resonance assignments derived from one- and two-dimensional homonuclear and heteronuclear NMR data (A). $^{19}$F NMR spectra of the photoproduct: (B) $^1$H-decoupled, (C) $^1$H-decoupled selectively on the resonances of H$_d$ (4.41 ppm) and (D) $^1$H-decoupled semi-selectively on both H$_c$ and H$_m$ (4.83 ppm). The observed $^1$H-$^{19}$F coupling constants confirm the stereochemistry of the photoproduct; the $^3$J$_{H-F}$-coupling constants in the range of 20 Hz and 6 Hz are characteristic of cis- and anti-orientations, respectively. The cis-orientation of H$_c$ and H$_d$ is also confirmed by their J-coupling constant ($^3$J$_{Hc-Hd}$ = 10.1 Hz; the coupling constants fall in the range of 10 Hz and 4 Hz for cis- and anti-orientations for cyclobutanes, respectively). The $^1$H and $^{19}$F NMR data were collected on 400- and 600-MHz spectrometers, respectively.

Figure S6: Expansions of $^1$H NMR of fbpcu
The overlaid $^1$H-$^1$H COSY (A) and $^1$H-$^{13}$C HSQC (B) cross-sections of the photoproduction.

The representative resonances and cross peaks are marked according to the numbering scheme shown in Figure S6.
Figure S9.

The overlaid $^1$H-$^{13}$C HMBC/HSQC cross-sections of the photoproduct. The HMBC and HSQC peaks are shown in black and red respectively; note that there is no HSQC peak observed for the carbon attached to fluorine.
Figure S10.

A \(^1\text{H}-13\text{C}\) HSQC/HMBC overlaid cross-section of the photoproduct showing the correlations of pyridyl and carbonyl carbons. HSQC cross peaks are colored red, whereas HMBC peaks are colored black. The cross peaks of only the tertiary and carbonyl carbons are marked.
5) Mass Spectrometry

Figure S11. HRMS of fbpcu.

**Single Mass Analysis**
- Tolerance = 3.0 mDa / DBE: min = -1.5, max = 100.0
- Element prediction: Off
- Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
404 formula(e) evaluated with 3 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-120 H: 0-200 N: 0-8 O: 0-5 F: 0-1

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<th>mDa</th>
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**References**

4. in *SDBSWeb*, National Institute of Advanced Industrial Science and Technology, Tokyo, Japan.