Ball Milled Glyco-graphene oxide conjugates markedly disrupted *Pseudomonas aeruginosa* **biofilm.**

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Example of numeration

Scheme S1. a) TMSOTf, DCM, r.t., 1h, 82% yield $(1:1.8 \alpha:\beta$ mixture); b) K₂CO₃, methanol, r.t., 1h, 90% yield; c) H2, Pd(OH)² 20% on carbon, MeOH:AcOEt 1:3, r.t., 1h, quantitative yield.

Scheme S2. a) TMSOTf, DCM, r.t., 1h, 40% yield; b) K_2CO_3 , methanol, r.t., 1h, 94% yield; c) H_2 , Pd(OH)₂ 20% on carbon, MeOH:AcOEt 1:3, r.t., 1h, 97% yield.

Scheme S3. a) TMSOTf, DCM, r.t., 1h, 82% yield; b) K_2CO_3 , methanol, r.t., 1h, 98% yield; c) H_2 , Pd(OH)₂ 20% on carbon, MeOH:AcOEt 1:3, r.t., 1h, 94% yield.

Synthesis of 9. To an ice-cooled solution of **7** (0.996 g, 2.02 mmol) and **8** (700 mg, 4.0 mmol) in dry dichloromethane (16 mL), trimethylsilyl trifluoromethanesulfonate (73 µL, 0.40 mmol) was added. The reaction mixture was stirred at r.t. for 1 h, then it was neutralized with triethylamine (167 µL, 1.2) mmol). Thus, pyridine (268 µL, 3.33 mmol), anhydride acetic (340 µL, 3.33 mmol) and 4dimethylaminopyridine (82 mg, 0.2 mmol) were added. The reaction mixture was stirred at r.t. for 1h, then it was diluted with dichloromethane (150 mL) and washed with a saturated solution of NH4Cl (3 x 15 mL) and Brine (1 x 15mL). The organic phase was anhydrificated with Na2SO4, filtered and concentrated under vacuum. The crude was purified by flash chromatography on silica gel to give **9** as β-anomer (523 mg, 64%) as yellow oil. β-anomer: $[\alpha]_{25}^D = -54.6^\circ$ (*c* = 0.42, CHCl₃); ¹H-NMR $(400 \text{ MHz}, \text{CDCl}_3, \delta)$: 5.21 (dd, $J_{4.5} = 0.8 \text{ Hz}, J_{3.4} = 3.6 \text{ Hz}, 1H, H-4$), 5.17 (dd, $J_{1.2} = 8.0 \text{ Hz}, J_{2.3} =$ 10.4 Hz, 1H, H-2), 5.50 (dd, *J3-4* = 3.6 Hz, *J2-*³ = 10.4 Hz, 1H, H-3), 4.51 (d, *J1-2* = 8.0 Hz, 1H, H-1), 3.98 – 3.93 (m, 1H, H-7A), 3.81 – 3.77 (m, 1H, H-5), 3.73 – 3.69 (m, 1H, H-7B), 3.67 – 3.62 (m, 8H, H-8, H-9, H-10, H-11), 3.38 (at, *J11-12* = 5.2 Hz, 2H, H-12), 2.16 (s, 3H, CH3), 2.04 (s, 3H, CH3), 1.21 (s, 3H, CH3), 1.21 (d, *J5-6* = 6.4 Hz, 3H, H-6). ¹³C-NMR (100 MHz, CDCl3, δ): 101.15, 71.32, 70.71, 70.68, 70.37, 70.28, 70.01, 69.12, 68.93, 68.90, 50.67, 20.77, 20.67, 20.61, 16.03; MS (ESI) *m/z***:** $[M+Na]^+$ calcd for $C_{18}H_{29}N_3O_{10}Na^+$ 470.18; found 470.14; NMR data are in agreement with the literature**.** [1]

Synthesis of 10. To a dispersion of 9 (561 mg, 1.25 mmol) in methanol (5 mL), potassium carbonate (52 mg, 0.38 mmol) was added. The reaction mixture was stirred at r.t. for 1 h, then it was diluted with methanol and neutralized with a solution of HCl (1% in methanol). The crude was filtered on silica gel to give 10 (362 mg, 90%) as a yellow oil. $[\alpha]_{25}^D = -28.2^\circ$ ($c = 0.35$, CD₃OD); ¹H-NMR (400 MHz, CD3OD, δ): 4.23 (d, *J1-2* = 7.2 Hz, 1H, H-1), 3.99 – 3.93 (m, 1H, H-7A), 3.72 – 3.59 (m, 11H, H-4, H-5, H-7B, H-8, H-9, H-10, H-11), 3.48 – 3.47 (m, 2H, H-2, H-3), 3.39 (at, *J11-12* = 5.2 Hz, 2H, H-12), 1.25 (d, *J5-6* = 6.4 Hz, 3H, H-6). ¹³C-NMR (50 MHz, CD3OD, δ): 103.44, 73.60, 71.58, 70.87,

70.54, 70.11, 69.99, 69.65, 68.11, 50.33, 15.24; MS (ESI) m/z : [M+Na]⁺ cacld for C₁₂H₂₃N₃O₇Na⁺ 344.14; found 344,20. NMR data are in agreement with the literature.[1]

Synthesis of 4. To a solution of 10 (268 mg, 0.85 mmol) in methanol: ethyl acetate 1:3 (11 mL) in H₂ atm, Pd(OH)² 20% on carbon (178 mg, 0.25 mmol) was added. The reaction mixture was stirred at r.t. in H_2 atmosphere for 1 h, then it was filtered on Celite⁵²¹ and concentrated to give 4 (292 mg, quantitative yield) as a yellow oil. $[\alpha]_{25}^D = 69^\circ$ (*c* = 0.5, CH₃OH); ¹H-NMR (400 MHz, CD₃OD, δ): 4.26 (d, *J1-2* = 7.5 Hz, 1H, H-1), 3.74-3.72 (m, 1H, H-4), 3.70-3.67 (m, 10H, H-7, H-8, H-9, H-10, H-11), 3.62-3.60 (m, 1H, H-5), 3.49-3.46 (m, 1H, H-2), 3.31-3.29 (m, 2H, H-12), 1.26 (d, *J6-5* = 6.6 Hz, 3H, H-6). ¹³C-NMR (100 MHz, CD3OD, δ): 103.44, 74.45, 71.23, 70.81, 70.57, 69.95, 69.01, 68.67, 39.29, 15.61. MS (ESI) m/z : [M+H]⁺ calcd for C₁₂H₂₆NO₇⁺ 296.17; found 296.15. NMR data agree with the literature.^[1]

Synthesis of 12. To an ice-cooled solution of **11** (1.27 g, 2.6 mmol) and **8** (910 mg, 5.2 mmol) in dry dichloromethane (16 mL), trimethylsilyl trifluoromethanesulfonate (60 µL, 0.39 mmol) was added. The reaction mixture was stirred at r.t. for 1 h, then it was neutralized with triethylamine (167 µL, 1.2) mmol). Thus, pyridine (321 µL, 3.99 mmol), anhydride acetic (373 µL, 3.99 mmol) and 4dimethylaminopyridine (115 mg, 0.52 mmol) were added. The reaction mixture was stirred at r.t. for 1h, then it was diluted with dichloromethane (150 mL) and washed with a saturated solution of NH4Cl $(3 \times 15 \text{ mL})$ and Brine $(1 \times 15 \text{ mL})$. The organic phase was anhydrificated with Na₂SO₄, filtered and concentrated under vacuum. The crude was purified by flash chromatography on silica gel to give **12** as β-anomer (523 mg, 40%) as yellow oil. $[α]^{D_{25}} = +137°$ ($c = 0.30$, CHCl₃); ¹H-NMR (400 MHz, CHCl3, δ): 5.37 (ad, *J* = 3.4 Hz, 1H, H-4), 5.19 (dd, *J2-3* = 10.5 Hz, *J2-1* = 8.0 Hz, 1H, H-2), 5.00 (dd, *J3-2* = 10.5 Hz, *J3-4* = 3.4 Hz, 1H, H-3), 4.56 (d, *J1-2* = 8.0 Hz, 1H, H-1), 4.18-4.08 (m, 2H, H-6), 3.97- 3.92 (m, 1H, H-7A), 3.90 (at, J = 6.7 Hz, 1H, H-5), 3.76-3.71 (m, 1H, H-7B), 3.67-3.65 (m, 8H, H-8, H-9, H-10, H-11), 3.39-3.37 (m, 2H, H-12), 2.13 (s, 3H, CH3), 2.05 (s, 3H, CH3), 2.03 (s, 3H, CH3), 1.97 (s, 3H, CH3). ¹³C-NMR (100 MHz, CDCl3, δ): 170.41, 170.27, 170.17, 169.49, 101.00, 71.10 70.80, 69.70, 69.05, 67.30, 61.47, 50.75, 20.22, 20.18, 20.14; MS (ESI) *m/z*: [M+Na]⁺ calcd for $C_{20}H_{31}N_3NaO_{12}$ ⁺ 528.18; found 528.24. NMR data are in agreement with the literature.^[1]

Synthesis of 13. To a dispersion of **12** (545 mg, 1.08 mmol) in methanol (6 mL), potassium carbonate (45 mg, 0.32 mmol) was added. The reaction mixture was stirred at r.t. for 1 h, then it was diluted with methanol and neutralized with HCl solution (1% in methanol). The crude was filtered on silica gel to give 13 (340 mg, 94%) as a yellow oil. $[\alpha]_{25} = +151^{\circ}$ ($c = 0.46$, CH₃OH); ¹H-NMR (400 MHz, CD3OD, δ): 4.25 (d, *J1-2* = 7.4 Hz, 1H, H-1), 4.03-3.99 (m, 1H, H-7A) 3.81 (ad, *J* = 3.2 Hz, 1H, H-4), 3.74-3.64 (m, 8H, H-8, H-9, H-10, H-11), 3.52-3.44 (m, 3H, H-6, H-7B), 3.39 – 3.36 (m, 2H, H-12). ¹³C-NMR (100 MHz, CD3OD, δ): 103.71, 75.34, 73.56, 71.19, 70.19, 70.08, 69.70, 68.76, 68.25,

61.16, 50.22. MS (ESI) m/z : [M+Na]⁺ calcd for C₁₂H₂₃N₃NaO₈⁺ 360.14; found 360.19. NMR data agree with the literature.^[2]

Synthesis of 5. To a solution of 13 (113 mg, 0.33 mmol) in methanol: ethyl acetate 1:3 (6 mL) in H₂ atm, Pd(OH)² 20% on carbon (70 mg, 0.1 mmol) was added. The reaction mixture was stirred at r.t. in H² atmosphere for 1 h, then it was filtered on Celite 521 and concentrated to give **5** (101 mg, 97%) as a yellow oil. $[\alpha]_{25}^D = 140^\circ$ (*c* = 0.38, CH₃OH); ¹H-NMR (400 MHz, CD₃OD, δ): 4.25 (d, J₁₋₂ = 7.4 Hz, 1H, H-1), 4.05-4.00 (m, 1H, H-7A), 3.81 (ad, *J* = 3.2 Hz, 1H, H-4), 3.74-3.62 (m, 11H, H-2, H-3, H-5, H-8, H-9, H-10, H-11), 3.56-3.44 (m, 3H, H-6, H-7B), 3.31-3.29 (m, 2H, H-12). ¹³C-NMR (100 MHz, CD3OD, δ): 103.72, 75.38, 73.57, 71.17, 70.12, 69.76, 68.99, 68.24, 61.20, 40.45; MS (ESI) m/z : [M+H]⁺ calcd for C₁₂H₂₆NO₈⁺ 312.17; found 312.20. NMR data agree with the literature.^[2] **Synthesis of 15.** To an ice-cooled solution of **14** (1.00 g, 2.03 mmol) and **8** (533 mg, 3.04 mmol) in dry dichloromethane (10 mL), trimethylsilyl trifluoromethanesulfonate (37 µL, 0.20 mmol) was added. The reaction mixture was stirred at r.t. for 1 h, then it was neutralized with triethylamine (85 μ L, 0.61 mmol). Thus, pyridine (245 μ L, 3.04 mmol), anhydride acetic (282 μ L, 3.04 mmol) and 4dimethylaminopyridine (49 mg, 0.2 mmol) were added. The reaction mixture was stirred at r.t. for 1h, then it was diluted with dichloromethane (150 mL) and washed with saturated solutions of NH4Cl $(3 \times 15 \text{ mL})$ and Brine $(1 \times 15 \text{ mL})$. The organic phase was anhydrificated with Na₂SO₄, filtered and concentrated under vacuum. The crude was purified by flash chromatography on silica gel to give **15** (820 mg, 82%) as pure α-anomer as yellow oil. $[\alpha]_{25}^D = 21^\circ$ ($c = 0.337$, CHCl₃); ¹H-NMR (400 MHz, CDCl3, δ): 5.36 (dd, *J2-3* = 3.6 Hz, *J3-4* = 10.0 Hz, 1H, H-3), 5.28 (at, *J4-3* = 9.6 Hz, 1H, H-4), 5.26 (dd, *J1-2* = 2.0 Hz, *J2-3* = 3.6 Hz, 1H, H-2), 4.87 (d, *J1-2* = 1.6 Hz, 1H, H-1), 4.31 – 4.26 (m, 1H, H-6_a), 4.14 – 4.03 (m, 2H, H-6_b, H-5), 3.85 – 3.78 (m, 1H, H-7A, CH₂), 3.70 – 3.64 (m, 9H, H-7B, H-8, H-9, H-10, H-11), 3.39 (at, *J* = 5.2 Hz, 2H, H-12), 2.15 (s, 3H, CH3), 2.10 (s, 3H, CH3), 2.03 (s, 3H, CH3), 1.98 (s, 3H, CH3). ¹³C-NMR (100 MHz, CDCl3, δ): 97.68, 70.77, 70.67, 70.04, 69.58, 69.07, 68.43, 67.39, 66.20, 62.43, 50.67, 20.83, 20.64; MS (ESI) *m/z*: [M+Na]⁺ calcd for $C_{20}H_{31}N_3NaO_{12}$ ⁺ 528.18; found 528.17. NMR data agree with the literature.^[1]

Synthesis of 16. To a dispersion of 15 (530 mg, 1.04 mmol) in methanol (4.2 mL), potassium carbonate (43 mg, 0.31 mmol) was added. The reaction mixture was stirred at r.t. for 1 h, then it was diluted with methanol and neutralized with a HCl solution (1% in methanol). The crude was filtered on silica gel to give 16 (345 mg, 98%) as a yellow oil. $[\alpha]_{25}^D = 27.3^\circ$ ($c = 0.333$, CH₃OH); ¹H-NMR (400 MHz, CD3OD, δ): 4.79 (d, *J1-2* = 1.6 Hz, 1H, H-1), 3.86 – 3.80 (m, 3H, H-2, H-6a, H-7A, CH2), 3.72 – 3.53 (m, 13H, H-3, H-4, H-5, H-6b, H-7b, H-8, H-9, H-10, H-11), 3.37 (at, *J* = 4.8 Hz, 2H, H-12). ¹H-NMR (400 MHz, DMSO-d⁶, δ): 4.6 (d, *J*₁₋₂ = 1.6 Hz, 1H, H-1), 3.68 – 3.26 (m, 18H, H-2, H-3, H-4, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12). ¹³C-NMR (100 MHz, DMSO-d⁶, δ): 100.36, 74.31, 71.32, 70.69, 70.27, 70.09, 69.94, 69.70, 67.35, 66.12, 61.66, 50.50; MS (ESI) *m/z*: [M+Na]⁺ calcd for $C_{12}H_{23}N_3NaO_8^+$ 360.14; found 360.07. NMR data agree with the literature.^[2]

Synthesis of 6. To a solution of 16 (95 mg, 0.28 mmol) in methanol: ethyl acetate 1:3 (4 mL) in H_2 atm, Pd(OH)² 20% on carbon (59 mg, 0.08 mmol) was added. The reaction mixture was stirred at r.t. in H₂ atmosphere for 1 h, then it was filtered on Celite⁵²¹ and concentrated to give 6 (82 mg, 94%) as a yellow oil. $[\alpha]_{25}^D = 41.2^{\circ}$ (c = 0.412, CH₃OH); ¹H-NMR (400 MHz, CD₃OD, δ): 4.79 (d, J₁₋₂ = 1.6 Hz, 1H, H-1), 3.86-3.80 (m, 3H, H-3, H-4, H-7A), 3.70-3.62 (m, 9H, H-2, H-5, H-7B, H-8, H-9, H-10), 3.57-3.52 (m, 4H, H-6, H-11), 2.82 (at, *J* = 4.8 Hz, 2H, H-12). ¹³C-NMR (100 MHz, CD3OD, δ): 100.28, 73.19, 71.12, 70.98, 70.66, 70.12, 69.99, 69.85, 67.17, 66.24, 61.48, 40.41; MS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{12}H_{26}NO_8^+$ 312.17; found 312.15. NMR data agree with literature.^[2]

Thermogravimetric analysis (TGA).

Thermogravimetric analysis (TGA) was run in air on a ∼2 mg sample using a Q5000 IR model TA instrument (New Castle, UK) starting at 100 °C. The sample was kept isothermal for 60 min, then ramped up by 10 °C min−1 . Weight losses in the range 230-330 °C: Fuc-GO **1** (9.4%), Gal-GO **2** (11.4%), Man-GO **3** (9.6%), GObm (6.3%).

Figure S1 Thermogravimetric analysis (TGA) under air atmosphere of glyco-GO conjugates **1a**-**b** prepared in a 10 mL stainless-steel mixer mill and using two stainless-steel balls (ϕ = 1.0 cm) in wet grinding conditions (Fuc-GO **1a**) and in presence of an inert milling auxiliary (NaCl) in liquidassisted grinding conditions (Fuc-GO **1b**).

Figure S2 Thermogravimetric analysis (TGA) under air atmosphere of Gal compound **5**, Gal-GO **2** and **GObm** (pristine GO milled without monosaccharide derivatives).

Inductively coupled plasma atomic emission spectroscopy (ICP-AES).

Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used to determine the concentrations of iron (Fe), chromium (Cr), sulfur (S) and was performed in triplicate using a Varian 720-ES Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES). An accurately weighted amount of each sample was treated with a microwave-assisted digestion (CEM MARS Xpress) using 1.0 mL of suprapure HNO³ obtained by sub-boiling distillation and 1.0 mL of suprapure H2O2. Each sample was thus diluted to 10 mL with Ultrapure water (UHQ), spiked with 0.5 ppm of Ge used as an internal standard, and analyzed. Calibration standards were prepared by gravimetric serial dilution from commercial stock standard solutions of each element at 1000 mg L⁻¹. The wavelengths used for elements determination were: 238.204 nm for Fe, 267.716 nm for Cr, 182.562 nm for S whereas for Ge the line at 209.426 nm was used. The operating conditions were optimized to obtain maximum signal intensity, and between each sample, a rinse solution constituted of 2% v/v $HNO₃$ was used to avoid memory effects.

Table S1. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) analyses of glyco-GO conjugates **1**-**3.**

glyco-GO	$%$ Fe	$%$ Cr
Fuc-GO 1a	16.11	3.70
Fuc-GO 1b	13.11	2.04
Fuc-GO ₁	0.21	0.013
Gal-GO ₂	0.05	0.007
Man-GO 3	0.03	0.004

glyco-GO	$\bf C\%$	H%	$N\%$	$S\%$
Fuc-GO ₁	49.35	2.91	0.26	0.33
Gal-GO ₂	46.25	3.03	0.33	0.23
Man-GO 3	47.87	3.10	0.21	0.13

Table S2. Elemental analysis of glyco-GO conjugates **1**-**3.**

Infrared spectroscopy

The FT-IR measurements were carried out with the Shimadzu IRAffinity-1S instrument. Potassium bromide (KBr) pellets were prepared using 100 mg of potassium bromide and 1.0 mg of sample. The spectra were recorded under inert atmosphere and a pure potassium bromide pellet was used as background.

Figure S3. Infrared spectra of glyco-GO conjugates **1**-**3.**

UV-vis spectroscopy

The UV-Vis absorption measurements were carried out with the Varian Cary 4000 instrument. The samples were dispersed in water at a concentration of 0.5 mg/mL. Before the measurement, the dispersions were placed in an ultrasonic bath for 30 min.

Raman Spectroscopy

Raman spectra were collected using an inVia Qontor confocal Raman microscope (Renishaw). The 532 nm laser line was used, in combination with an 1800 L/mm grating. The selected objective was \times 50L (Leica). Spectra were acquired in the frequency range 700–2850 cm⁻¹, with an exposure time of 10s and 10% laser power.

NMR experiments

The solid-state NMR spectra were acquired in the CERM laboratory of the University of Firenze with a 16.47 T (700 MHz of proton Larmor frequency) Avance II Bruker instrument and a 20.0 T (850 MHz of proton Larmor frequency) Avance III Bruker instrument equipped, in both cases, with a 3.2 mm double resonance CP MAS probe. The MAS frequency was set to 20.0 kHz and temperature was set to 260 K to ensure an effective sample temperature around 298 K. In all the experiments 3.2 mm zirconia rotors with vespel caps were used. The reported 1D and 2D experiments were acquired at 20.0 T. In the 1D CP MAS experiment the ${}^{1}H \pi/2$ pulse was set to 2.5 µs, the CP contact time was 100 μ s, with a CP power level of 50 kHz on ¹³C and 78 kHz on ¹H. On ¹H a 70 to 100% linear ramp was used, and the power level refers to 100% of power. The ¹H decoupling was performed during the acquisition time with a SW_fTPPM decoupling sequence at 78 kHz of power. The recycle delay was set to 1.0 s and the acquisition time was 17.20 ms. The final spectrum was obtained summing up 40960 scans. The spectra were recorded with 1024 complex points and processed with 32768 complex points. Spectra were processed with an exponential widow function with 300 Hz of line broadening.

For the 2D 1 H- 13 C FSLG CP experiments (reported in figure S4) a standard Frequency-Switched Lee-Goldberg sequence was used. The $\pi/2$ proton pulse length and CP conditions were the same as reported above for 1D experiments. Same power and decoupling sequence were used also for the ¹H decoupling during the ^{13}C acquisition. For the FSLG ¹H homodecoupling, in the indirect dimension, a radiofrequency power of 100 kHz was used. The recycle delay was 1.0 s, the acquisition times were 17.07 ms for the ¹³C (direct) dimension and 1.176 ms for the ¹H indirect dimension. Up to 13312 scans were added, acquiring 1280 and 18 complex points in the direct and indirect dimension, respectively. The spectra were processed with a 2048x256 matrix of complex points using exponential widow function with 300 Hz of line broadening in both dimensions.

Figure S5. ¹³C MAS direct excitation solid-state NMR spectrum of the glyco-GO Gal-GO **2**. Spectra is acquired at 16.4 T (701.1 MHz of ¹H Larmor Frequency, 176.3 MHz of ¹³C Larmor frequency) and 12.0 kHz of MAS frequency. The resonance of the epoxide (C-O-C) and hydroxyl (C-OH) groups are clearly observed at about 60 and 70 ppm, respectively. Aromatic resonances of the C=C atoms are observed at about 129 ppm. An additional band around 160 ppm (that overlaps with a sideband around 190 ppm, denoted by *) can be attribute to carbonyl groups (C=O). The ¹³C $\pi/2$ pulse was set to 3.3 µs. The ¹H decoupling was performed during the acquisition time with a SW_fTPPM decoupling sequence at 78 kHz of power. The recycle delay was set to 10 s and the acquisition time was 15.36 ms. The final spectrum was obtained summing up 40960 scans. The spectra were recorded with 1536 complex points and processed with 4096 complex points. Spectra were processed with an exponential widow function with 200 Hz of line broadening. Spectra were acquired with 1024.

carbon-13 chemical shift (ppm)

Figure S6. ¹H–¹³C FSLG CP 2D spectrum of Gal-GO 2. The spectrum is acquired at 20.0 T (850 MHz of proton Larmor Frequency, 213.8 MHz of ¹³C Larmor frequency) and 20.0 kHz of MAS frequency. The ¹³C 1D spectrum of Gal-GO **2** is reported above the 2D map to help the analysis of correlations. Notably, the resonances of the galactose and the saccharide linker are easily recognized. The experimental details are reported above.

Table S3. Minimal bactericidal concentrations (MBC, lowest concentration of drug that kills more than 99% of the bacterial population) of glyco-GO conjugates **1**-**3**, pristine GO and GObm.

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