Electronic Supplementary Material (ESI) for Chemical Science.

Supporting Information

Fiber-Optic Array Using Molecularly Imprinted Microspheres for Antibiotic Analysis

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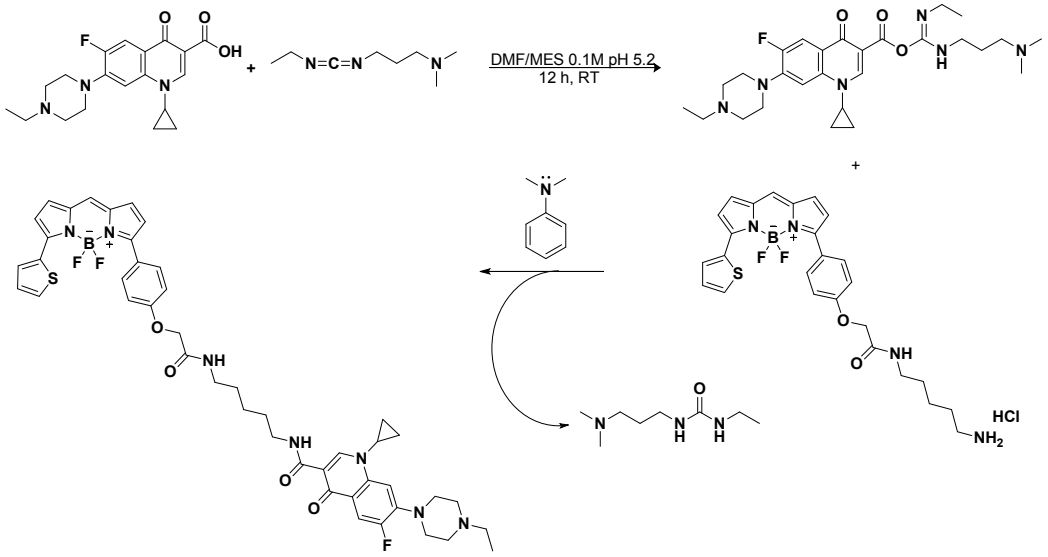
1. Synthesis of BODIFLOXACIN

The experimental procedure for the synthesis of BODIFLOXACIN is shown in Scheme S1. A solution of enrofloxacin (123 mg, 0.34 mmol) in 1 mL of DMF, was mixed with 600 µL of a fresh solution of 420 mg mL^{-1} EDC in 0.1 MES buffer (pH 5.7), and the product mixture was stirred in the dark at room temperature for 12 h to produce solution **1**.[S1] BODIPY® TR Cadaverine (5.0 mg, 0.009 mmol) was dissolved in 1 mL 0.1 M MES buffer (pH 5.7) and added to solution **1** with stirring to give solution **2**. Then, 227 mg (2.27 mmol) of DMAP was dissolved in 600 µL of DMF and 20 µL aliquots were added, in the dark, to solution **2** every 24 h. The product was isolated from the raw reaction mixture by semi-preparative HPLC (see below).



Scheme S1. Experimental procedure for the synthesis of BODIFLOXACIN.

2. Purification of BODIFLOXACIN by semi-preparative HPLC and spectroscopic characterization

The raw reaction mixture was completely transferred into a semi-preparative HPLC system: HP1200 LC from Agilent Technologies (Palo Alto, CA) equipped with a solvent delivery quaternary pump, an autosampler, diode array detector (DAD) and an analytical scale fraction

collector. (Experimental Section. Materials and Instrumentation). Chromatographic separation of BODIFLOXACIN from the precursor and synthesis-byproducts was achieved by use of a ZORBAX Eclipse XDB-C18 semipreparative column (250 mm x 9.4 mm, 5 μm) from Agilent Technologies (Palo Alto, CA). The flow rate of mobile phase was 5 mL/min and the detection wavelengths were 280 nm and 575 nm A gradient elution program was used with the mobile phase combining solvent A (MeCN), solvent B (H_2O with 0.1% TFA) and solvent C (MeOH) as shown in Table S1. The flow rate was 5 mL min^{-1}. BODIFLOXACIN eluted at 19.4 min, and the fractions were collected from 19.2 to 19.7 min.

Table S1. Gradient elution method used in the purification of BODIFLOXACIN.

Time (min)	MeCN (%)	H_2O with 0.1% TFA (%)	MeOH (%)
0	2	98	0
4	2	98	0
20	50	12	38
35	100	0	0

The solvent was evaporated at reduced pressure to afford a dark blue solid (1.98 g, 25% yield) that was identified as BODIFLOXACIN. High-resolution mass spectrum of purified BODIFLOXACIN was obtained with a time of flight mass spectrometer MALDI-TOF/TOF (Ultraflex, Bruker). ^{1}H-NMR and ^{13}C-NMR spectra were acquired on a Bruker Avance III 700 MHz using CD_3OD as solvent and tetramethylsilane (TMS) as internal standard.

^{1}H-NMR (700 MHz, CD_3OD, δ): 1.10 and 1.28 (4H, m, H-15 and H-16), 1.38 (3H, t, J=7.2 Hz, H-1), 1.40 and 1.46 (4H, m, H-21 and H-23), 1.64 (2H, m, H-22), 3.19-3.84 (15H, several m, H-

2, H-3, H-4, H-5, H-6, H-14, H-20 and H-21), 4.53 (2H, s, H-26), 6.77 (1H, d, J = 4.1 Hz, H-34), 6.89 (1H, d, J = 4.2 Hz, H-40), 6.94 (2H, d, J = 8.9 Hz, H-28 and H-29), 7.16 (1H, dd, J = 5.0 and 3.9 Hz, H-44), 7.21 (1H, d, J = 4.2 Hz, H-39), 7.25 (1H, d, J = 4.1 Hz, H-35), 7.45 (1H, d, J = 7.1 Hz, H-10), 7.49 (1H, s, H-37), 7.63 (1H, dd, J = 5.0 and 0.9 Hz, H-45), 7.88 (1H, J = 8.9 Hz, H-30 and H-31), 7.92 (1H, d, J = 13.1 Hz, H-9), 8.01 (1H, dd, J = 3.9 and 0.9 Hz, H-43), 8.74 (1H, s, H-17). ^{13}C-NMR (700 MHz, CD_3OD) δ: 8.5 (C-15 and C-16), 9.5 (C-1), 24.7 (C-23), 29.5 (C-22), 29.7 (C-21), 36.3 (C-14), 39.6 (C-20 and C-24), 48.2 (C-4 and C-6), 52.3 (C-3 and C-5), 53.2 (C-2), 68.1 (C-26), 107.7 (C-10), 111.6 (C-18), 113.2 (C-9), 115.5 (C-28 and C-29), 121.4 (C-40), 121.9 (C-34), 127.0 (C-32), 127.8 (C-37), 129.7 (C-44), 130.8 (C-45), 131.5 (C-39), 131.7 (C-35), 132.3 (C-30, C-31 and C-43), 135.2 (C-42), 138.0 (C-38), 138.4 (C-36), 139.9 (C-12), 148.4 (C-17), 151.5 (C-41), 159.6 (C-33), 160.3 (C-27), 166.9 (C-19), 170.6 (C-25), 176.7 (C-13). HRMS (MALDI-TOF/TOF): m/z calc. for $C_{45}H_{48}BF_3N_7O_4S$ $[M+H]^+$: 850.353; found: 850.351.

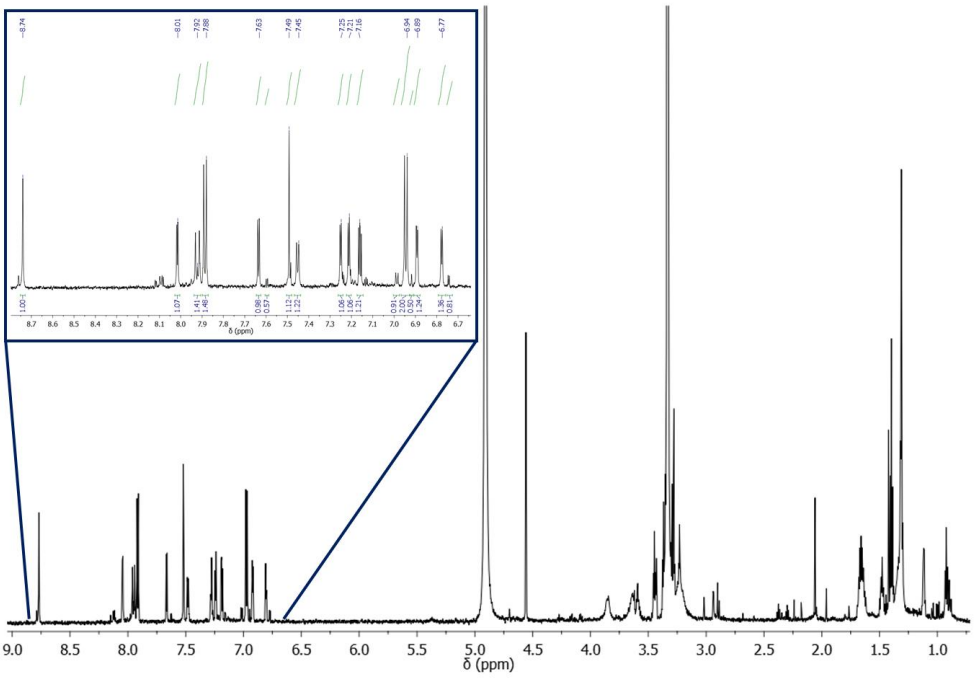


Figure S1. ^{1}H-NMR (700 MHz, CD_3OD) for BODIFLOXACIN.

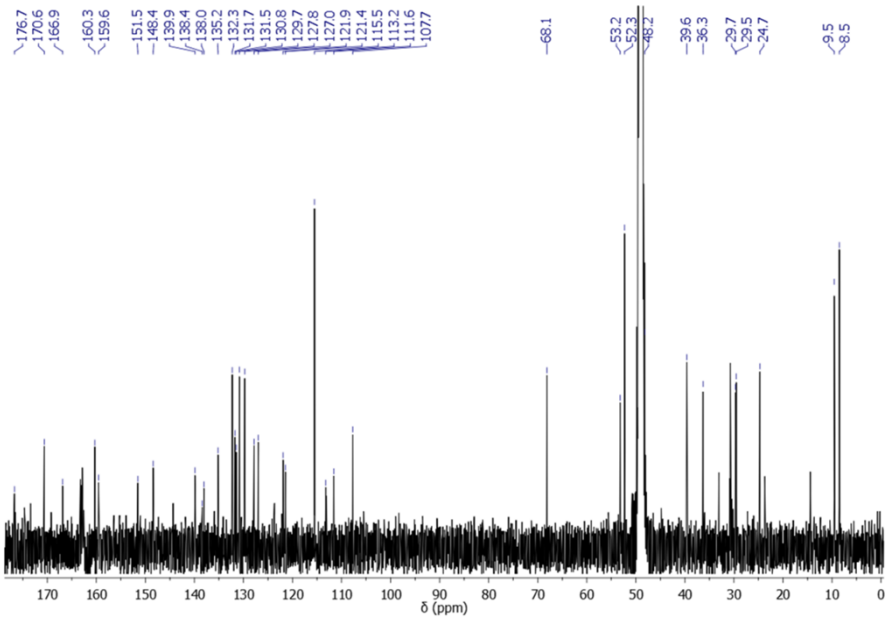


Figure S2. ^{13}C-NMR (700 MHz, CD_3OD) for BODIFLOXACIN.

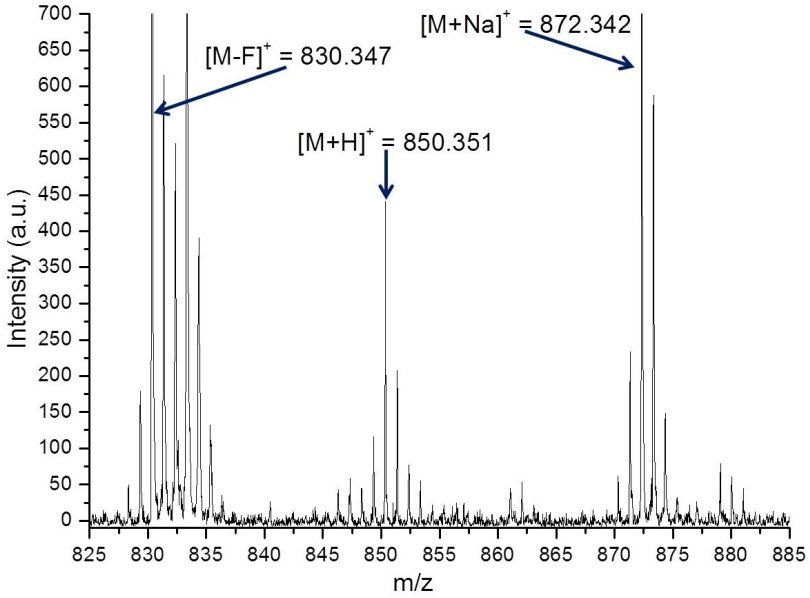


Figure S3. MALDI-TOF/TOF mass spectrum of BODIFLOXACIN.

Absorption spectra were recorded on a UV/VIS spectrophotometer (Cary 3 Bio, Varian) and fluorescence spectra were measured with a spectrofluorometer (FluoroMax 4, Horiba Scientific).

The fluorescence quantum yield, Φ_f, of BODIFLOXACIN in MeCN was calculated by the Parker and Rees method[S2] that relates the emission quantum yield of an unknown (BODIFLOXACIN) to that of a standard (Ox-170) fluorophore, the emission spectra of which have been recorded under the same conditions in optically diluted solution (A < 0.1):

$$\Phi_f^{Bodifloxacin} = \Phi_f^{Ox-170} \frac{A_{Ox-170}}{A_{Bodifloxacin}} \frac{S_{Bodifloxacin}}{S_{Ox-170}} \frac{n_{Bodifloxacin}^2}{n_{Ox-170}^2} \quad \text{(Eq. S1)}$$

where A is the absorbance of the solution at the excitation wavelength, S is the area under the fluorescence curve, and n is the solvent refractive index, in the case where the standard and the unknown have to be measured in different solvents ($n_{ethanol}$ = 1.3614; n_{MeCN} = 1.3441). Ox-170 in ethanol (Φ_f^{Ox-170} = 0.58 ± 0.03)[S3] was as reference fluorophore. Oxygen was removed from the solutions by argon purging for a minimum of 30 min before the measurements.

3. Chromatographic conditions

Chromatographic analysis was carried out with a HP-1100 series high performance liquid chromatography from Agilent Technologies (Palo Alto, CA, USA) consisting of a quaternary pump, on-line degasser, autosampler, column thermostat, automatic injector and a diode array (DAD) or a fluorescence detector (FAD). Chromatographic separations were performed with an Aqua C18 analytical column (250 mm x 4.6 mm, 5 μm) from Phenomenex (Torrance, CA). An isocratic program was used for ENRO and BODIFLOXACIN separation. The mobile phase consisted of a mixture of MeCN (with 0.5% TFA):water (with 0.5% of TFA) (26:74, *v/v*) at a flow rate of 1.0 mL min^{-1}. The retention times for BODIFLOXACIN and ENRO were 5.52 min and 7.05 min, respectively. Alternatively, for the cross-reactivity studies, chromatographic separation of the antibiotics was performed in the same isocratic conditions. All analyses were

carried out at a 1.0 mL min^{-1} flow rate and keeping the column temperature at 25 ºC. The fluorescence excitation/emission wavelengths were 280/440 nm for ENRO, CIPRO, NORF, FLUME and DANO, and at 575/620 nm for BODIFLOXACIN. Other interfering compounds such as, DOXY and PGP were detected by DAD at 350 nm and 280 nm, respectively. In all cases, quantification was performed using external calibration peak area measurements.

4. Binding of BODIFLOXACIN and ENRO to the encoded polymer microspheres

The affinity of the MIP and NIP microspheres for BODIFLOXACIN and ENRO was verified by equilibrium binding analyses. Different batches of MIPs and NIPs (2.5 mg) were shaken, at 70 rpm for 2.0 h, in solutions (1 mL) of 2 μM ENR, or BODIFLOXACIN, prepared in binary mixtures of MeCN and aqueous HEPES buffer (25 mM, pH 7.5) ranging from 50% ACN to 75% HEPES buffer. After incubation, suspensions were centrifuged at 3000 rpm for 2 min, and the concentration of ENRO and BODIFLOXACIN in the supernatant was measured by HPLC with FLD detection, as described previously.

The imprinting factor (IF) was calculated as B_{MIP}/B_{NIP}, where B is the amount of compound bound to the MIP/NIP polymers. All experiments were carried out in triplicate. The results are shown in Figure S4. The highest IF, both for BODIFLOXACIN and ENRO, was obtained at 50% (*v/v*) MeCN/HEPES buffer (25 mM, pH 7.5) that was selected for assay development.

To evaluate the optimum concentration of BODIFLOXACIN for the ENRO assay, a fixed amount of MIP (0.25 mg) was incubated, with shaking at 70 rpm for 2 h, with increasing concentrations of BODIFLOXACIN in the absence, and in the presence of a constant concentration of ENRO (20 μM), in 1 mL of MeCN:HEPES 25 mM pH 7.5 (50:50, *v/v*) as the solvent.[S4,S5] A control experiment was carried out using the NIP in place of the MIP. The

microparticles were centrifuged and the concentration of BODIFOXACIN in the supernantant was monitored by HPLC-FLD.

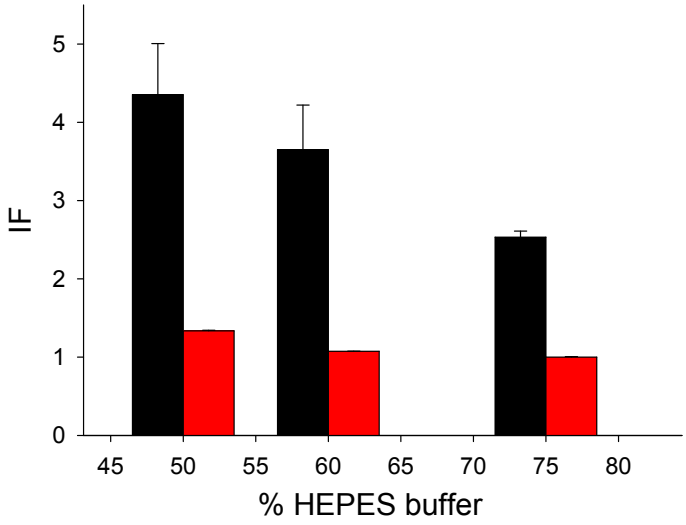


Figure S4. Imprinting factors for ENRO (black) and BODIFLOXACIN (red) as a function of the composition of the incubation solvent, ACN:HEPES buffer (25 mM, pH 7.5). Incubation time: 120 min; 2.5 mg mL^{-1} polymer; [ENRO] = [BODIFLOXACIN] = 2 μM. Each data point represents the average of three replicate measurements.

As shown in Figure S5, BODIFLOXACIN competes with ENRO for the polymeric binding sites, as the labeled ENRO is displaced from the MIP in the presence of the template molecule. The largest difference in the fraction of BODIFLOXACIN bound to the polymer, in the presence ($B_{ENRO20\ \mu M + Bodifloxacin}$) and absence ($B_{Bodifloxacin}$) of ENRO, between the MIP and the NIP, were obtained when using 250 nM BODIFLOXACIN. This value was thus selected for the development of the competitive assay.

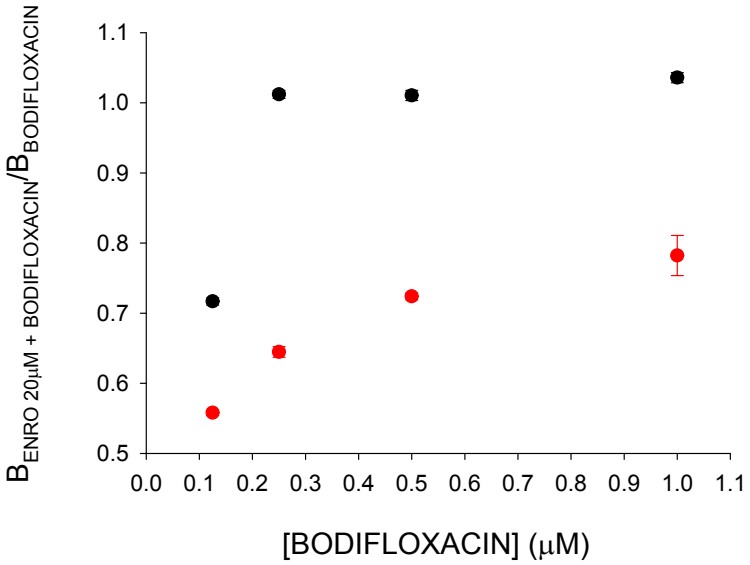


Figure S5. Fraction of BODIFLOXACIN bound to a constant amount of MIP (black) and NIP (red) microspheres (0.25 mg) in 1 mL of 50:50 (*v/v*) MeCN:HEPES 25 mM pH 7.5, as a function of the labelled ENRO concentration (0.1 μM to 1.0 μM), after 2 h incubation in the absence ($B_{BODIFLOXACIN}$) and in the presence of 20 μM ENRO ($B_{ENRO20\mu M\ +\ BODIFLOXACIN}$). Each data point represents the average of three replicate measurements.

5. Image acquisition and data analysis

Table S2. Optical filter information and exposure time for the BODOFLOXACIN, C30 and $[Ru(dip)_3]Cl_2$ channels.

Dye	Interference filter (nm)	Dichroic mirror (nm)	Cut-off (nm)	Exposure time (s)
BODIFLOXACIN	550	600	590	2
C30	405	414	425	3
$[Ru(dip)_3]Cl_2$	470	490	500	1

The average signal intensity on each microsphere type, was calculated using the tri-mean criterion instead of conventional mean statistic. The tri-mean is the weighted average of the distribution median and its two quartiles as defined in Eq S2, where Q_1, Q_2, and Q_3 denote the 25%, 50% and 75% points in a distribution, respectively.

$$trimean = \frac{Q_1 + 2Q_2 + Q_3}{4} \qquad (Eq.\ \text{S2})$$

References

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