Electronic supplementary information

Chemical analysis using 3D printed glass microfluidics

Eran Gal-Or[a]*,Yaniv Gershoni[a], Gianmario Scotti[b], Sofia M. E. Nilsson[b], Jukka Saarinen[b], Ville Jokinen[b,c], Clare J. Strachan[b], Gustav Boije af Gennäs[b], Jari Yli-Kauhaluoma[b] and Tapio Kotiaho[b,d]*

[a]MICRON3DP Ltd., IMC Group – ISCAR, Atir Yeda 21, Kfar-Saba 4464316, Israel

[b]Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, P.O. Box 56 (Viikinkaari 5 E), FI-00014, Finland

[c]Department of Chemistry and Materials Science, School of Chemical Engineering, P.O. Box 13500 (Aalto University), FI-00076 Aalto, Finland.

[d]Department of Chemistry, Faculty of Science, University of Helsinki, P.O. Box 55, FI-00014, Finland

*Correspondence to: eran.galor@micron3dp.com and tapio.kotiaho@helsinki.fi

Supplementary information

The following items are included: Cleaning of the microreactor; Cleaning of the direct infusion device channel; Figures S1 – S9; Linezolid synthesis and associated mass spectra with different reagent flow rates; Offline mass spectrometry experiments: reagents for the offline MS, reaction conditions in the offline MS, offline MS measurement conditions; MS^n mass spectra and fragmentation schemes; Electrospray ionization (ESI) mass spectra measured with the direct infusion device; Validation of the purity of (5*S*)-5-(aminomethyl)-3-[3-fluoro-4-(4-morpholinyl)phenyl]-1,3-oxazolidin-2-one and linezolid (commercial reference compound): LC-MS analysis for the validation, ^{1}H NMR analysis for validation; Movies; Reference.

Cleaning of the microreactor

After each reaction study, in total 10 mL of a 5 vol% aqueous hydrogen peroxide (prepared from the 30% w/w hydrogen peroxide solution and purified water) solution was infused into the additively manufactured glass microreactor through two of the reactor inlets, using two 5 mL syringes (ID 10.3 mm, Hamilton Bonaduz AG, Bonaduz, Switzerland) and the flow rate 5 µL/min per syringe (PHD 2000 syringe pump, Harvard Apparatus, Holliston, USA). This solution was left in the reactor for at least 12 h, then the reactor was flushed with at least 2 mL purified water and finally at least with 2 mL of acetonitrile. These cleaning steps were performed in a similar manner to the cleaning with hydrogen peroxide solution. The 5 mL syringes were connected to the fittings and unions (IDEX Europe GmbH, Erlangen, Germany) on the deactivated fused silica capillaries glued to the inlets of the reactor. All the capillaries, except the ones through which the cleaning liquid was infused (same capillaries as used for infusing the reactant solutions in the preceding online experiment, thus inlet/outlet 1 and 2 in early experiments and inlet/outlet 4 and 5 in the later experiments when inlet/outlet 1 was plugged with epoxy) and the one plugged with epoxy (inlet/outlet 1) in later experiments, of the reactor were open during the cleaning procedure.

Cleaning of the direct infusion device channel

The direct infusion device was cleaned using the following procedure. First, the channel was emptied (if it was not already empty) by removing the liquid with a syringe and flushing with nitrogen. Then methanol:water 80:20 + 1 vol% formic acid was infused into the channel, the channel was emptied as described above, and then re-filled with methanol:water 80:20 + 1 vol% formic acid. This procedure was repeated until no intensive peaks of the analytes could be seen in the mass spectrum.

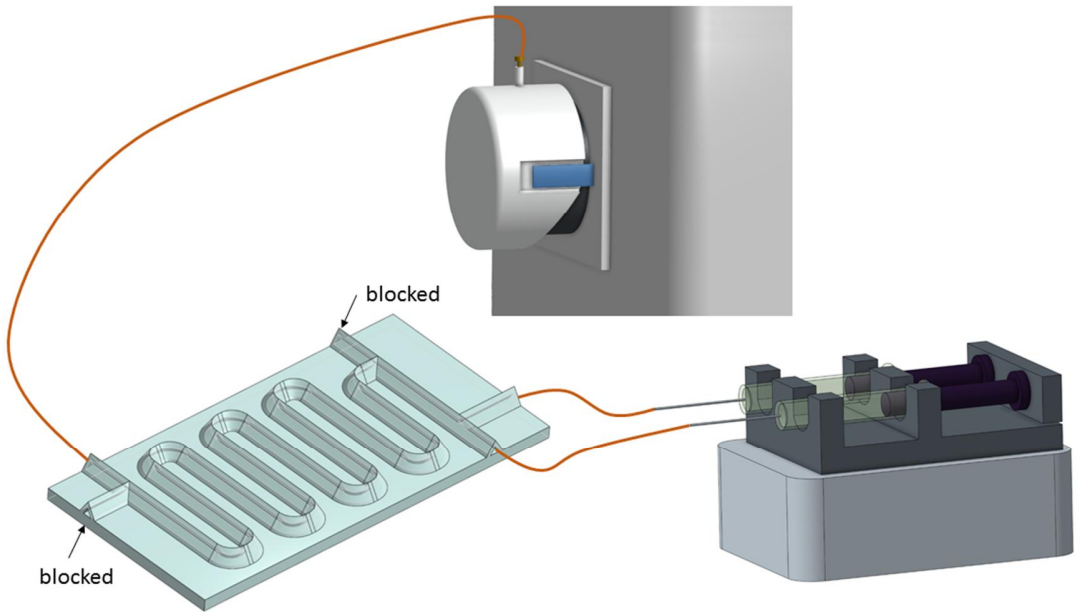


Figure S1. Schematic picture of the set-up used in the mass spectrometry reaction experiments.

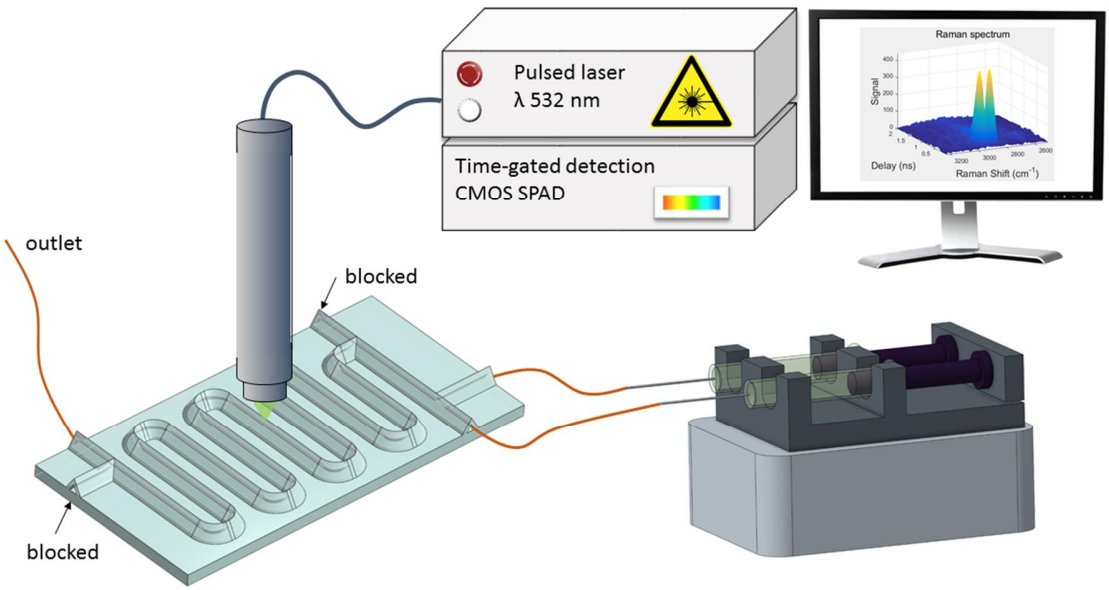


Figure S2. Schematic picture of the set-up used in the Raman spectroscopy measurements.

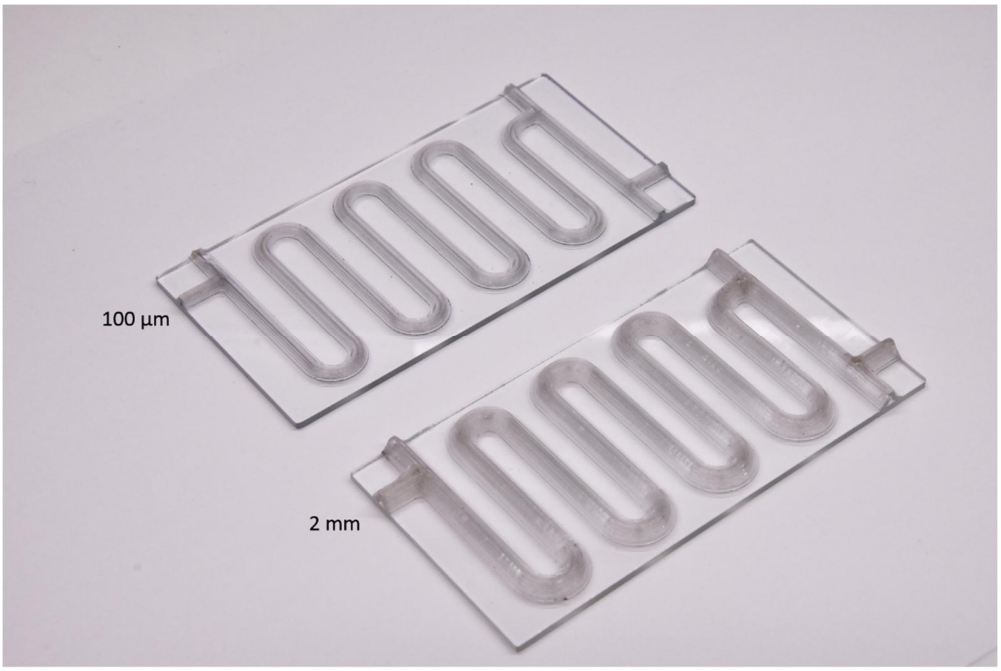


Figure S3. Microreactor devices with the small (marked using 100 µm, cross-sections pictures of this microreactor in Figures 2c, 2f and S4) and large channel cross-sections (marked using 2 mm, cross section pictures of this microreactor in Figure 2e and Figure S7). Fluidic functionality and leak tightness of these devices was not tested. Brightness and contrast of the image have been edited to increase visibility of the additively manufactured glass devices.

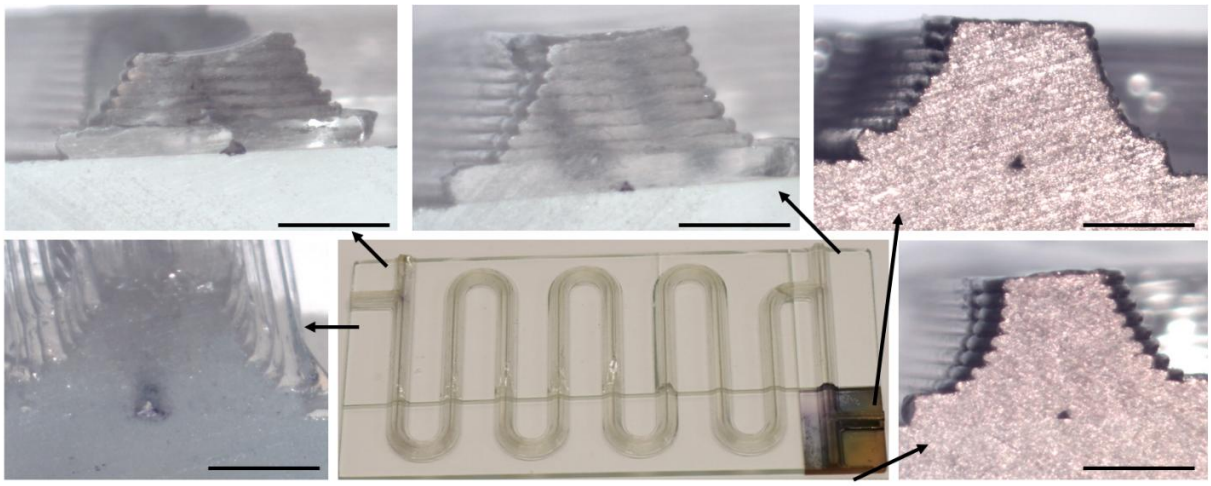


Figure S4. The shape and size of the channels in the small channel cross-section device in Figure S3. The black dipstick (1 mm) in the optical channel end micrographs is shown only to demonstrate size range of the channels (for more precise dimensions, see the SEM picture in Figure 2f). Brightness and contrast of the device image have been edited to increase visibility of the additively manufactured glass device.

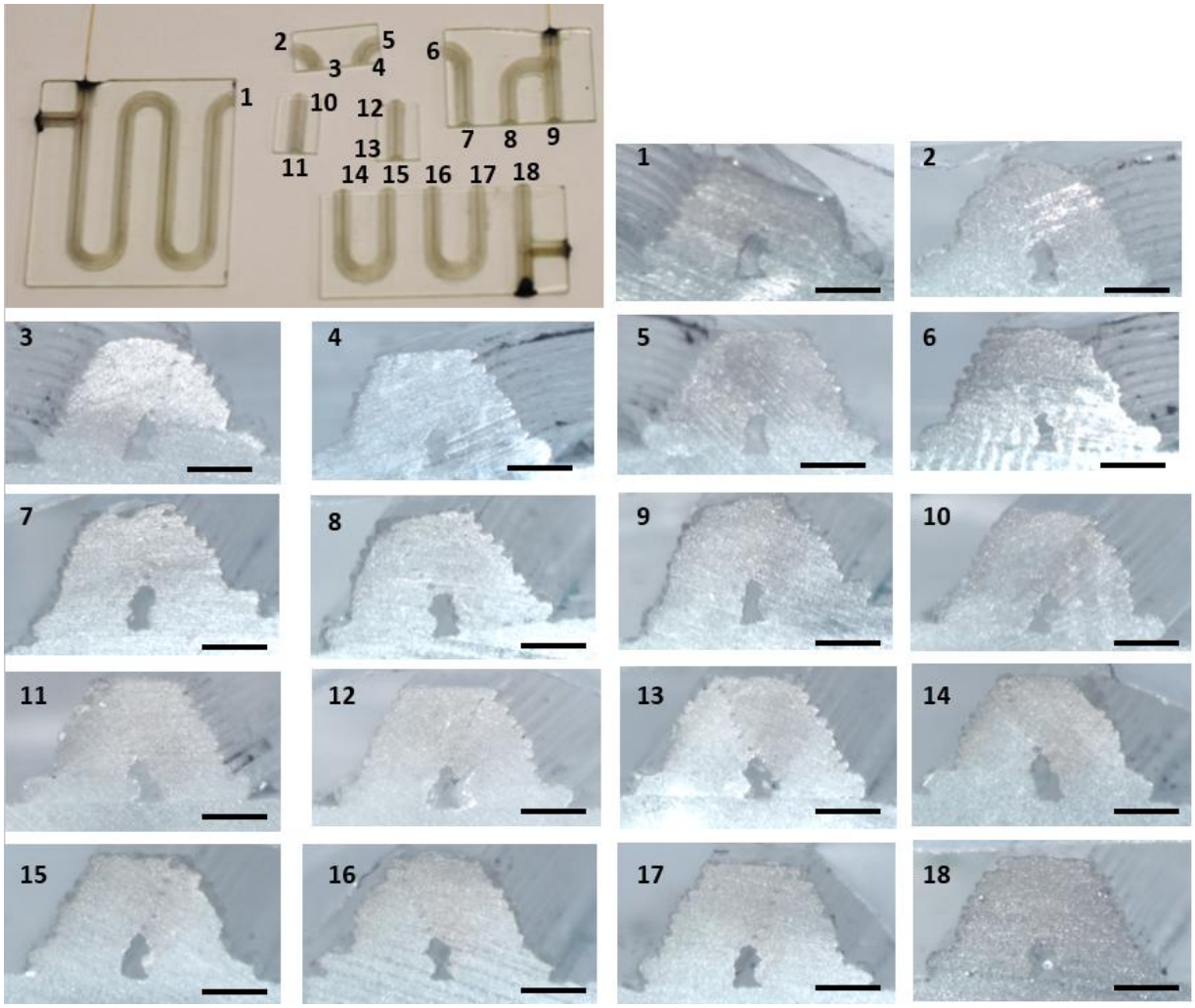


Figure S5. The size and shape of the channels in the mid-size channel cross-section device in Figure 2a. The black dipstick (1 mm) in the optical channel end micrographs is shown only to demonstrate size range of the channels (for more precise dimensions, see the SEM picture in Figure 2g and S6). Positions of the pieces containing channel ends 10-13 can be other way round also. Brightness and contrast of the glass pieces image have been edited to increase visibility of the pieces.

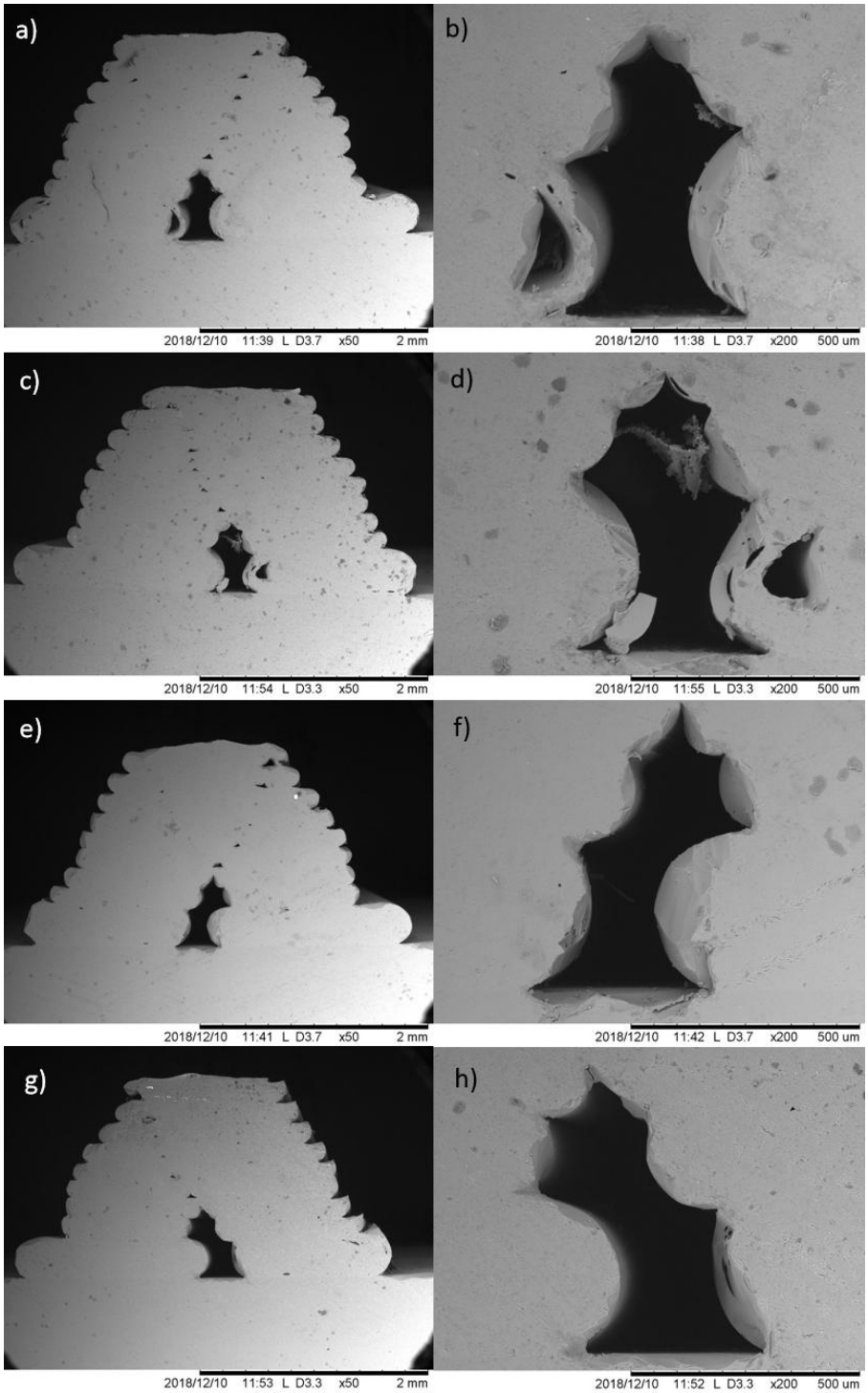


Figure S6. SEM-pictures to show the size and shape of the channel ends 10-13 (see position in the device from Figure S5, positions of the pieces containing channel ends 10-13 can be other way round also) in the mid-size channel cross-section device in Figure 2a.

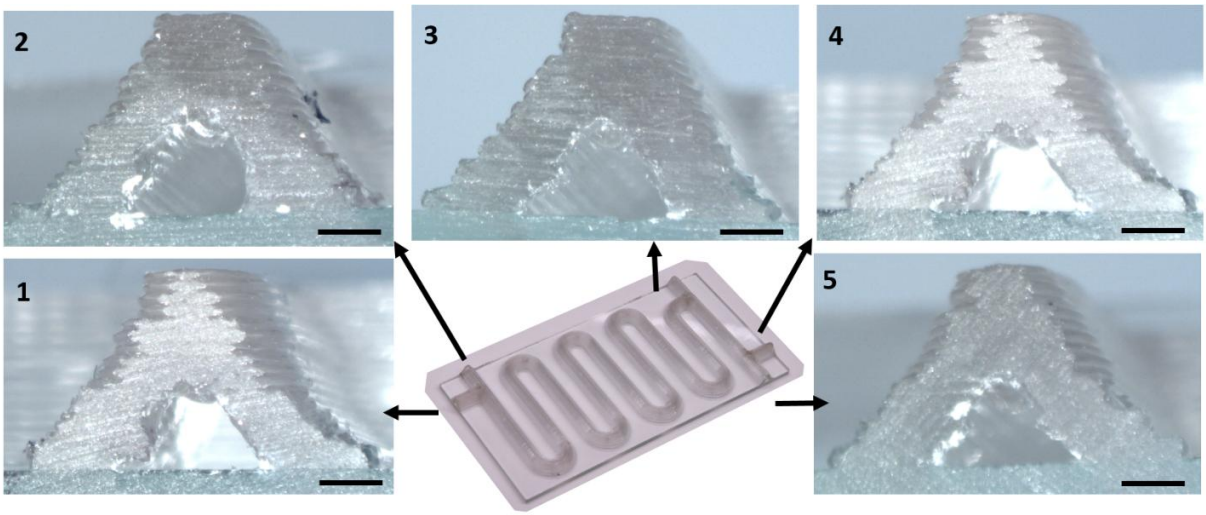


Figure S7. The size and shape of the channels in the large channel cross-section device in Figure S3. The black dipstick (1 mm) in the optical channel end micrographs is shown only to demonstrate size range of the channels. Brightness and contrast of the device image have been edited to increase visibility of the additively manufactured glass device.

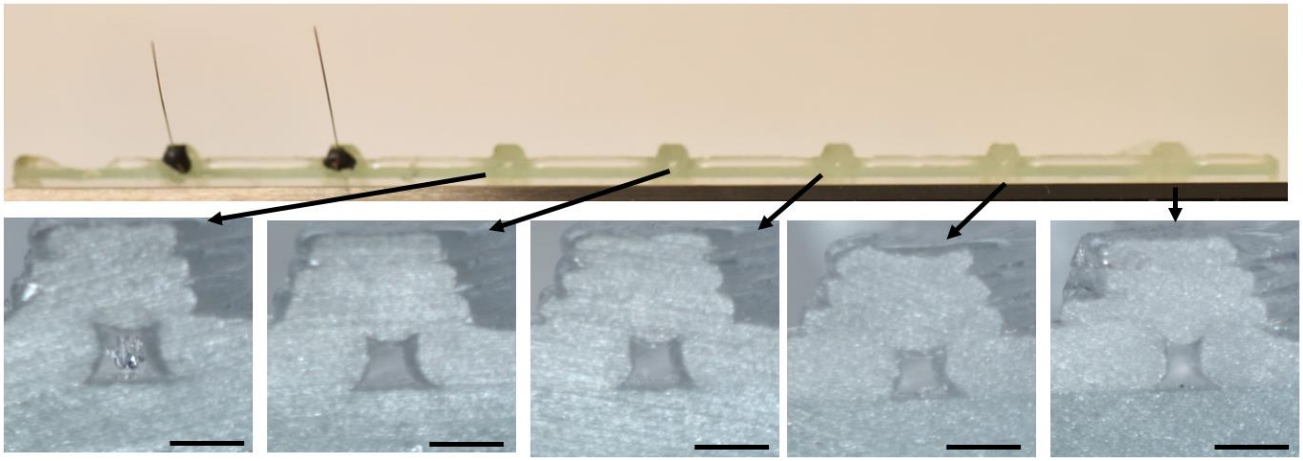


Figure S8. The size and shape of the channels in the direct infusion device (Figure 2b and the top picture). The black dipstick (1 mm) in the optical channel end micrographs is shown only to demonstrate size range of the channels. Brightness and contrast of the device image have been edited to increase visibility of the additively manufactured glass device.

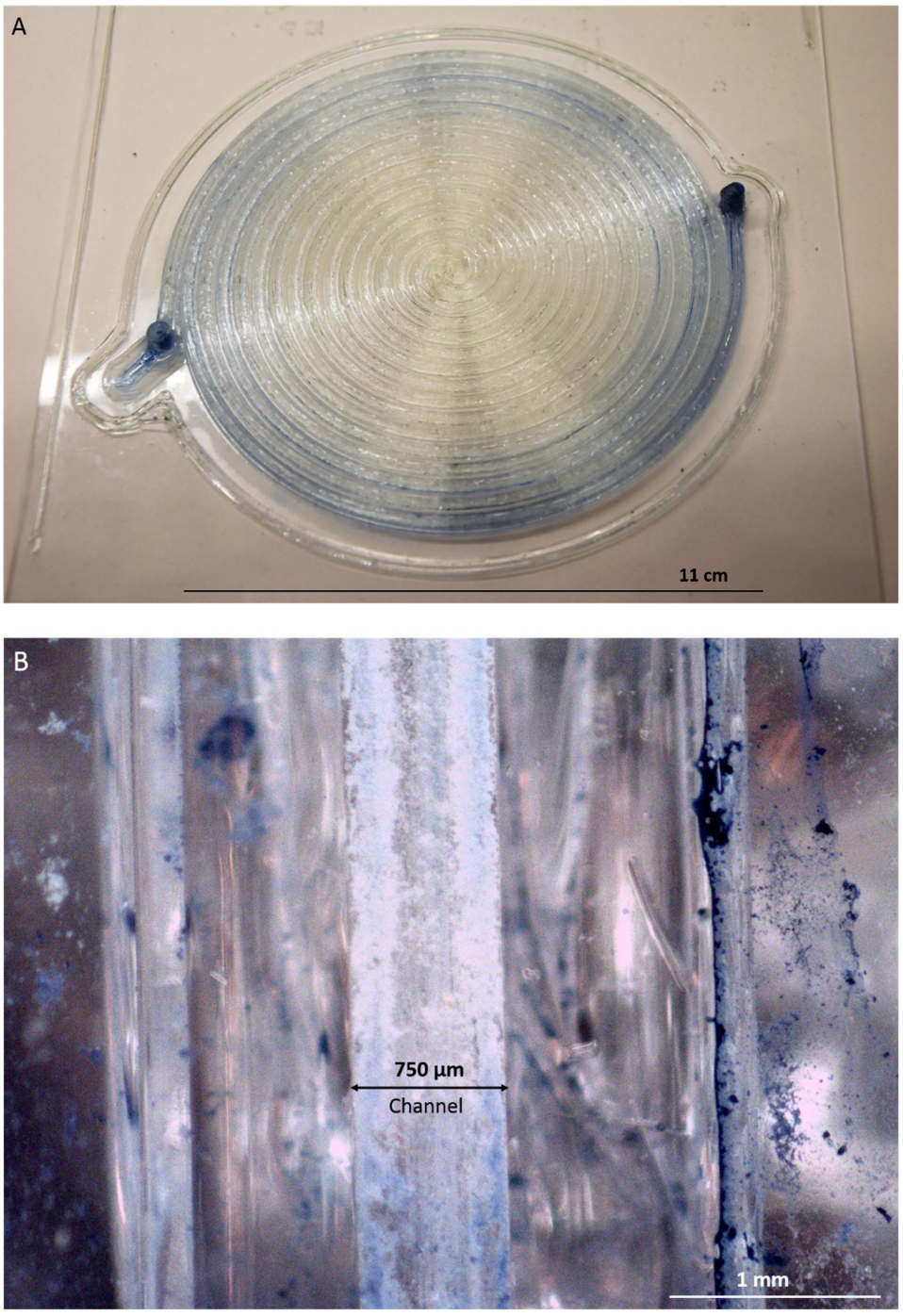
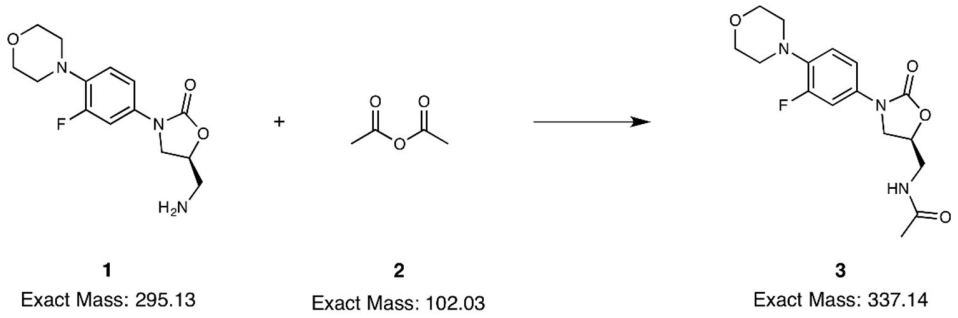


Figure S9. The spiral device. (a) Photograph of a spiral device with single inlet and outlet, which are connected by a 2.5 m long channel, and (b) a photograph demonstrating the channel dimension (taken from the glass base plate side). The dipsticks are shown to demonstrate size range. Brightness and contrast of the image (a) have been edited to increase visibility of the additively manufactured glass device.

Linezolid synthesis and associated mass spectra with different reagent flow rates



Scheme S1. The reaction between (5*S*)-5-(aminomethyl)-3-[3-fluoro-4-(4-morpholinyl)phenyl]-1,3-oxazolidin-2-one (**1**) and acetic anhydride (**2**) to produce linezolid (**3**).

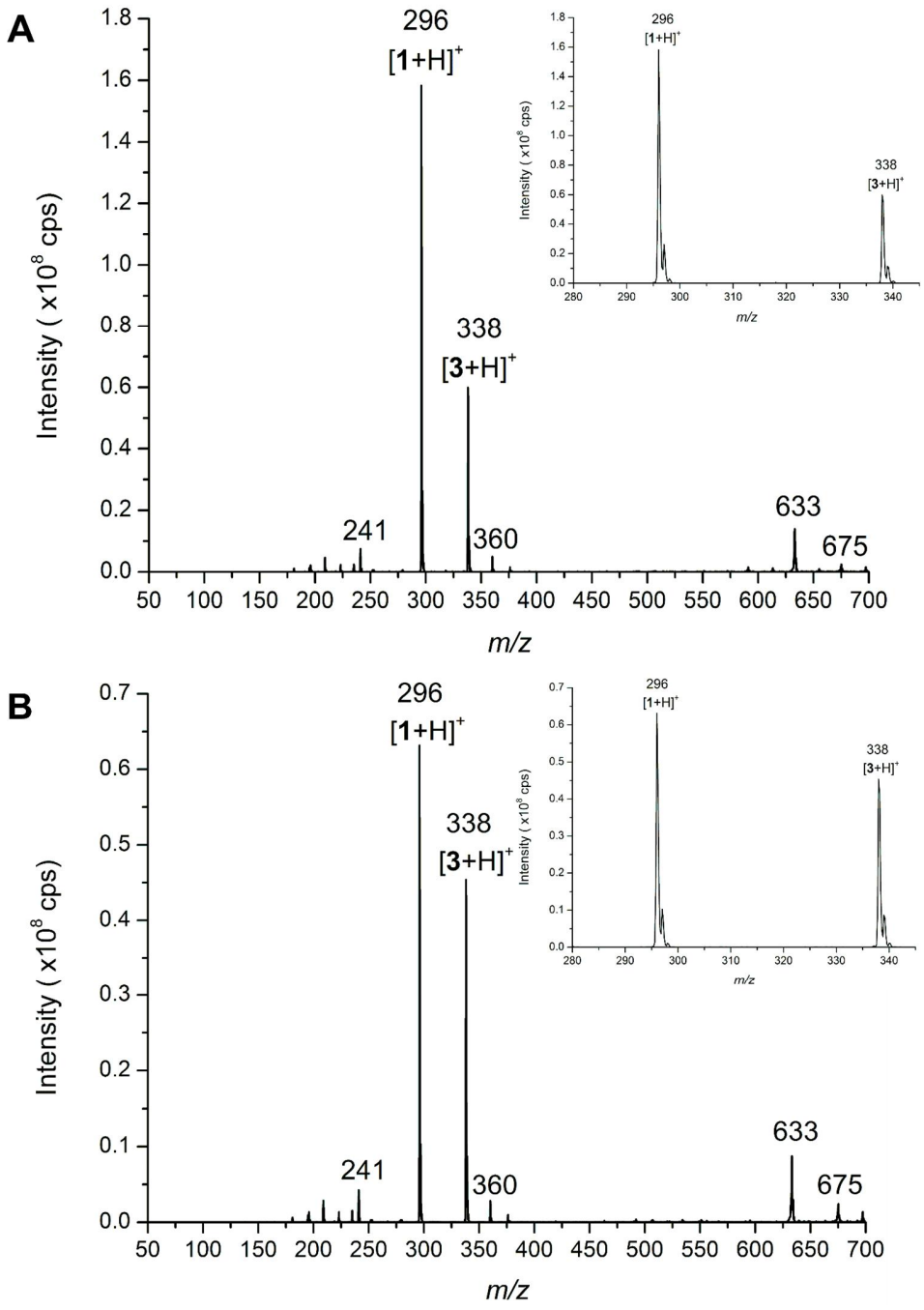


Figure S10. Mass spectra averaged over 1 min at two different reactant infusion flow rates. (a) 4 μL/min per reactant (total flow 8 μL/min), and (b) 750 nL/min per reactant (total flow 1.5 μL/min). The insets show the same spectra as the full *m/z* scan range spectra, but only in the *m/z* range where the analytes of interest ([**1**+H]$^+$, at *m/z* 296 and [**3**+H]$^+$, at *m/z* 338) are present.

Offline mass spectrometry experiments

Offline mass spectrometric experiments were performed to study the formation of linezolid as a function of the reaction time to support the online reaction studies using the additively manufactured glass microreactor.

Reagents for the offline MS

The same chemicals, obtained from the same suppliers, as for the online MS experiments were used in the offline MS experiments: (5*S*)-5-(aminomethyl)-3-[3-fluoro-4-(4-morpholinyl)phenyl]-1,3-oxazolidin-2-one (also called linezolid related compound C, **1** in Figure 3a and Scheme S1, ≥97% purity) was obtained from Carbosynth Ltd. (Compton, UK), acetic anhydride (≥99% pure, compound **2**, in Figure 3a and Scheme S1) was obtained from Sigma-Aldrich (Steinheim, Germany) and the sample was prepared in Chromasolv®-grade acetonitrile (Honeywell, Morris Plains, USA).

Reaction conditions in the offline MS

A 1 mL sample of 50 µM (5*S*)-5-(aminomethyl)-3-[3-fluoro-4-(4-morpholinyl)phenyl]-1,3-oxazolidin-2-one and 50 µM acetic anhydride was prepared in acetonitrile in an amber 2 mL screw cap vial which was sealed with a 9-mm blue PTFE/RS screw cap (both from Agilent Technologies, Santa Clara, USA) at room temperature. The contents of the vial was manually shaken, and a sample at reaction times ~0, 10, 20, 30, 40, 50, 60 and 110 min was taken using the ASAP-method (an atmospheric solids analysis probe, ASAP) as described below.

Offline MS measurement conditions

An Advion Expression^S CMS quadrupole mass spectrometer (Advion Inc., Ithaca, USA) operated in positive mode atmospheric pressure chemical ionization was used for the offline measurements. The temperature of the ion source capillary was 200 °C, the ion source capillary voltage was 120 V, and the atmospheric pressure chemical ionization corona discharge used was 5 µA. Nitrogen heated to 350 °C was used as the nebulizer gas and introduced with the flow rate 8 L/min. The MS scan range 50-700 *m/z* was used, and the scan time was 500 ms. The sample to be analyzed was introduced into the mass spectrometer with an atmospheric solids analysis probe (ASAP) equipped with a borosilicate glass capillary (cleaned between measurements), dipped into the offline

reaction mixture. Before inserting the probe with the sample into the inlet of the mass spectrometer, the glass capillary was wiped with a Kimtex wipe. Acquisition of mass spectra was started some seconds before the sample was introduced, and continued for at least 30 s (depending on how long time the analyte signals were present). The time interval when the intensity of the extracted ion profiles (EIPs) of the analyte ions (*m/z* 296 and 338) were clearly increased were averaged to obtain mass spectra (thus at least for 8 scans and 12 s). The presented mass spectrum in Figure S11 contains 11 scans and is averaged over 18 s. The obtained data was processed with the software Data Express (Advion Inc., Ithaca, USA). The peak areas for the plot in Figure S12 were obtained from the EIPs of the ions of interest (*m/z* 296 and 338) at different reaction times. The peak area ratio (%) of *m/z* 338 and *m/z* 296 at these time points (~0, 10, 20, 30, 40, 50, 60 and 110 min) are plotted as a function of the reaction time.

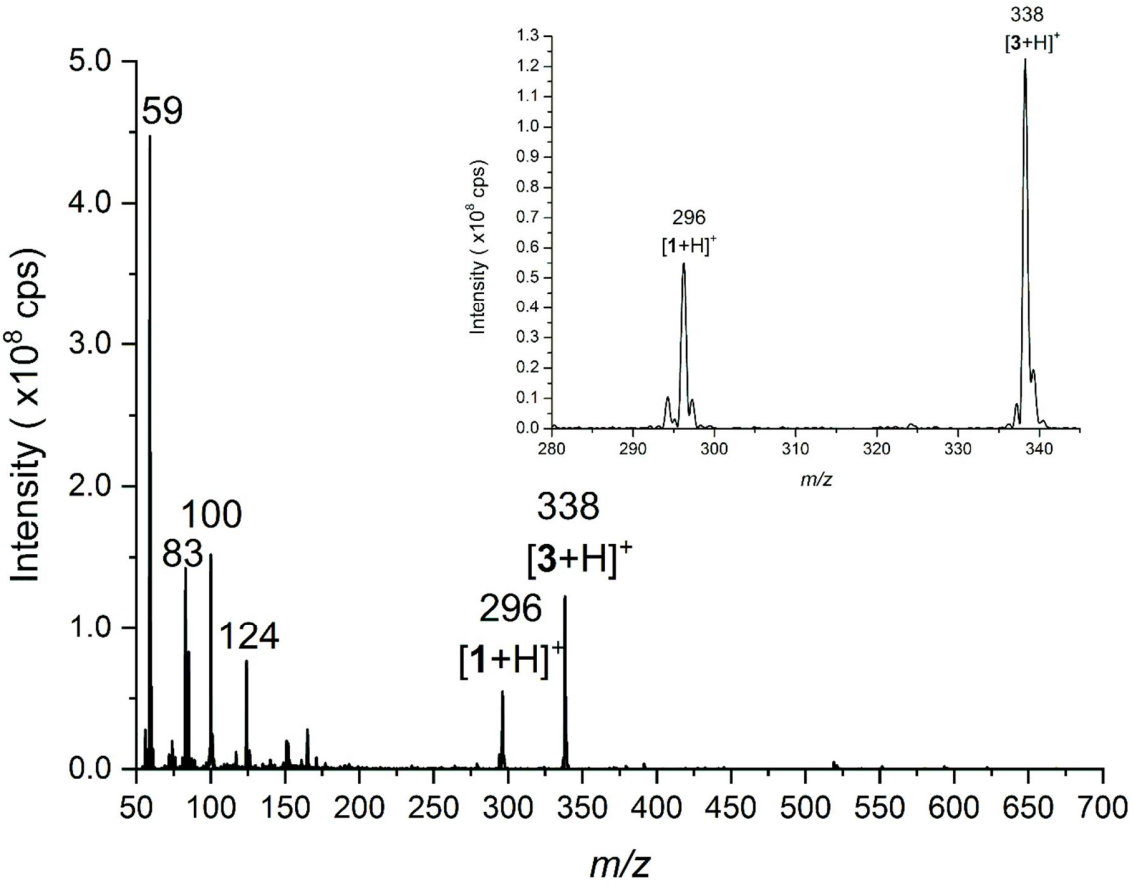


Figure S11. A mass spectrum recorded with the quadrupole mass spectrometer after 40 min offline reaction time, averaged over 18 s (11 scans). The inset shows part of the mass spectrum in the *m/z* range where the analytes of interest ([**1**+H]$^+$, at *m/z* 296 and [**3**+H]$^+$, at *m/z* 338) are present.

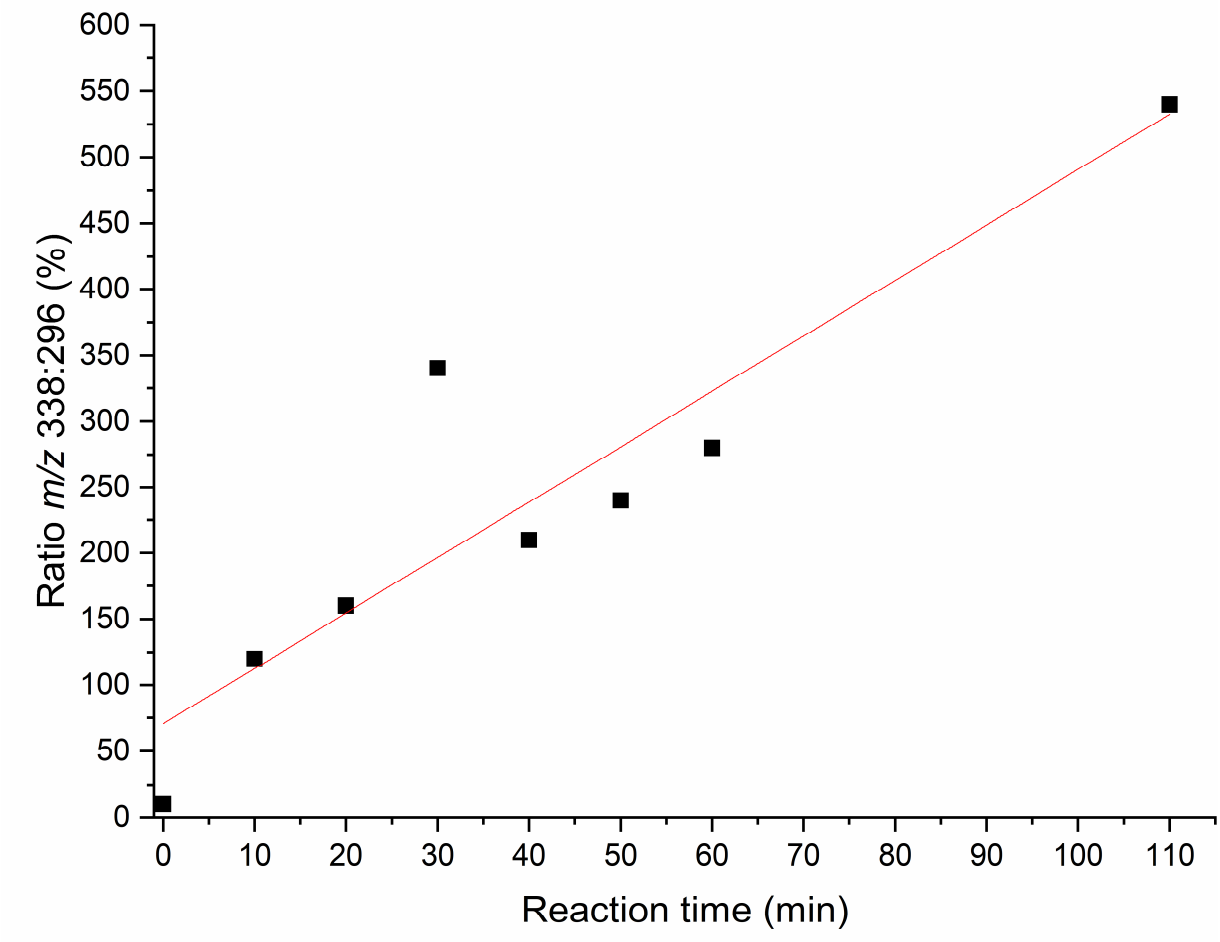


Figure S12. A plot of the ratio (%) of the peak area of *m/z* 338 ([**3**+H]$^+$) divided by the peak area of *m/z* 296 ([**1**+H]$^+$) as a function of the reaction time.

MS^n mass spectra and fragmentation schemes

To confirm the identities of the ions *m/z* 296 and 338 seen in the mass spectra presented for the reaction solution (Figure 3b and S10), product ion (MS/MS) mass spectra (Figure S13a and S14a, respectively) for them were measured and compared to the ones recorded for pure (5*S*)-5-(aminomethyl)-3-[3-fluoro-4-(4-morpholinyl)phenyl]-1,3-oxazolidin-2-one and linezolid (Figure S13b and S14b). Good correlation was observed and the fragment ions observed for the protonated linezolid in our study agree well with the *m/z* values reported earlier.[1] Additionally, the good correlation between the MS/MS mass spectrum of the precursor ion *m/z* 296 ([**1**+H]$^+$) measured for the ion observed in the reaction solution (Figure S13a) and the MS^3 spectrum of the precursor ion *m/z* 296 formed as a fragment of the precursor ion *m/z* 338 ([**3**+H]$^+$, Figure S15a) also supports the interpretation of the identities of these ions seen in the reaction mixture mass spectrum. In the Schemes S2 and S3 the proposed fragmentation routes of the precursor ions *m/z* 296 and 338 are presented.

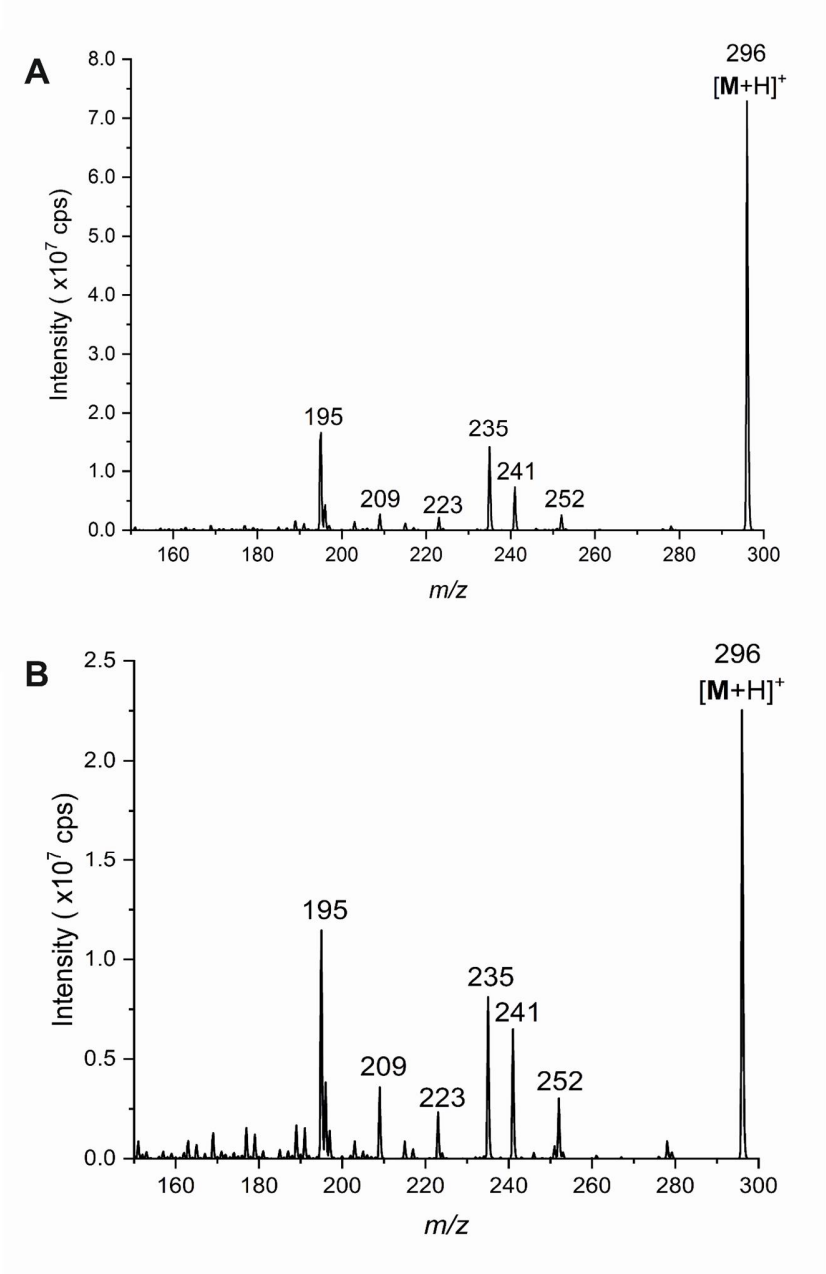


Figure S13. Product ion mass spectra of the precursor ion *m/z* 296 recorded with the ion trap mass spectrometer. (a) Product ion mass spectrum recorded when the reaction was conducted online in the additively manufactured glass microreactor and the fragmentation amplitude 0.35 V was used. (b) Product ion mass spectrum recorded offline, by directly infusing (flow rate 5 µL/min) a sample of 170 µM (5*S*)-5-(aminomethyl)-3-[3-fluoro-4-(4-morpholinyl)phenyl]-1,3-oxazolidin-2-one and fragmenting the precursor ion with the fragmentation amplitude 0.4 V.

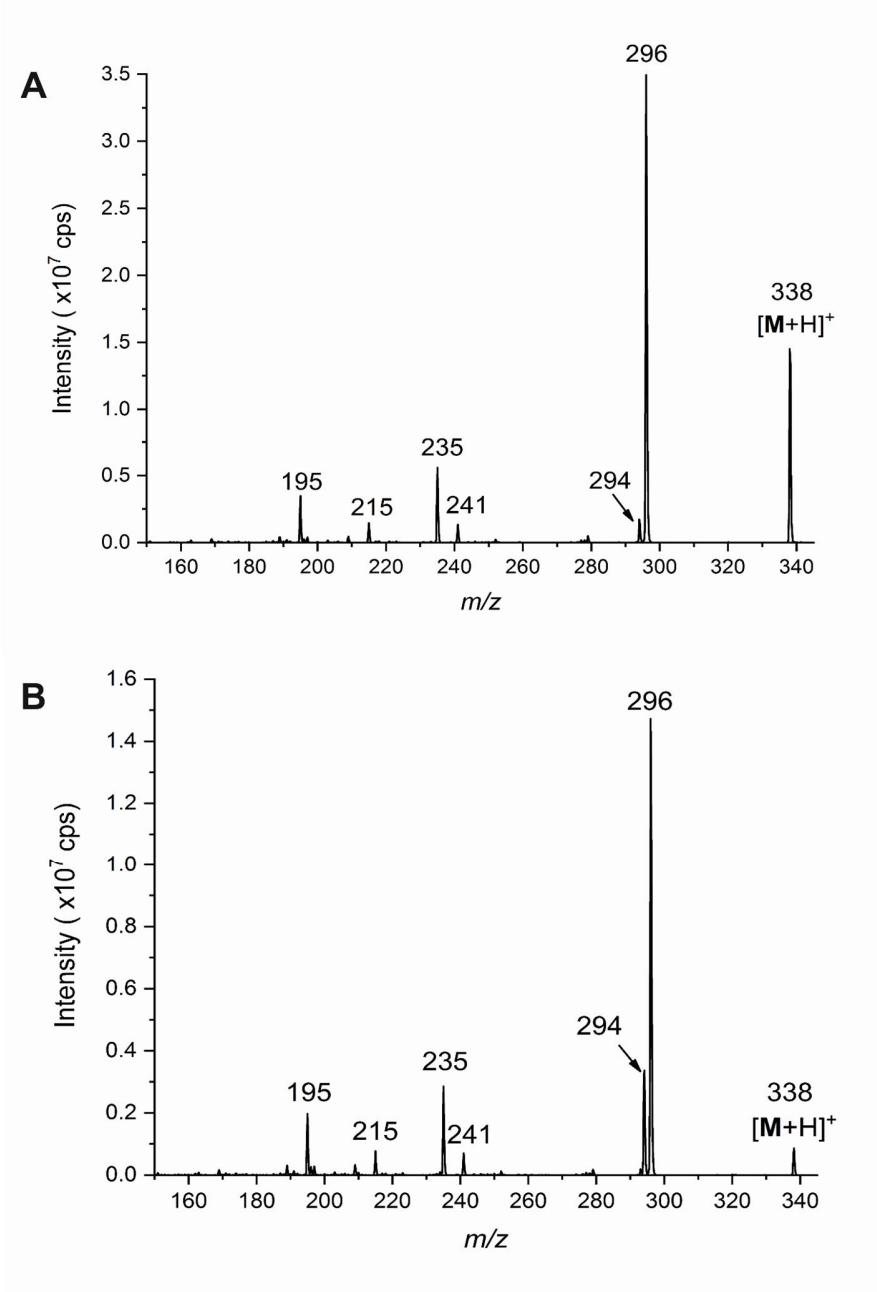


Figure S14. Product ion mass spectra of the precursor ion *m/z* 338 recorded with the ion trap mass spectrometer**.** (a) Product ion mass spectrum recorded when the reaction was conducted online in the additively manufactured glass microreactor and the fragmentation amplitude 0.35 V was used. (b) Product ion mass spectrum recorded offline, by directly infusing (flow rate 5 µL/min) a sample of 50 µM linezolid and fragmenting the precursor ion with the fragmentation amplitude 0.45 V.

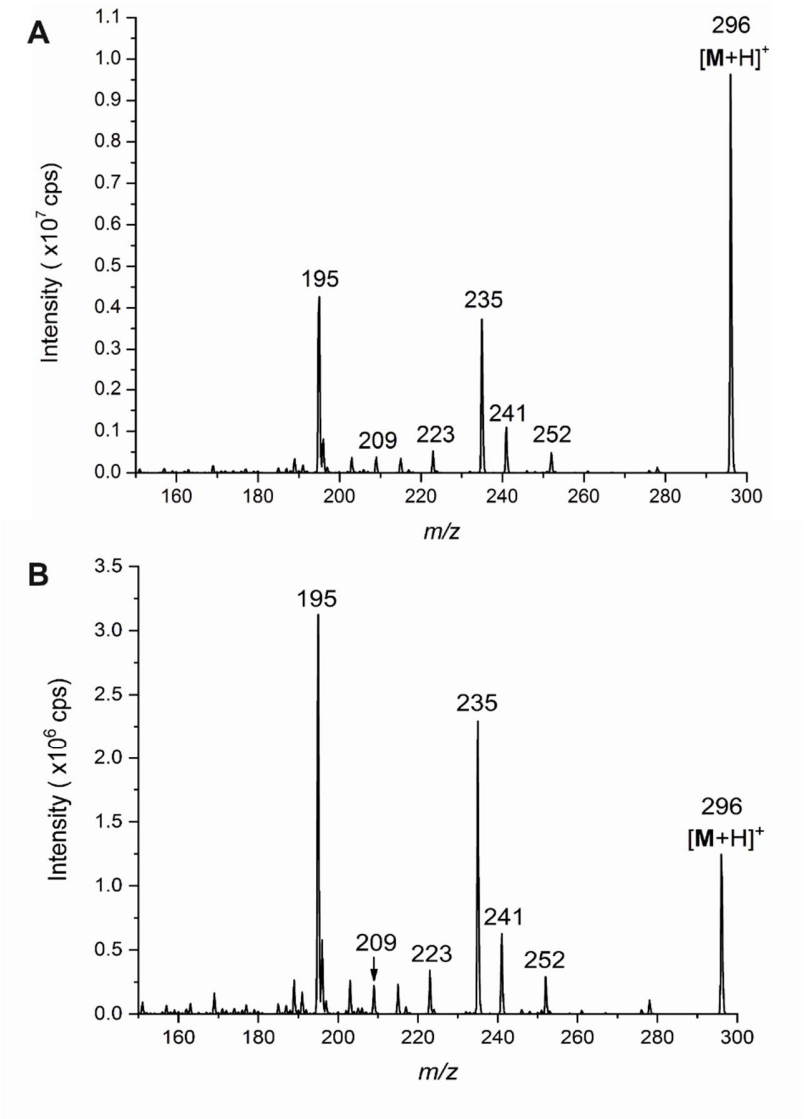
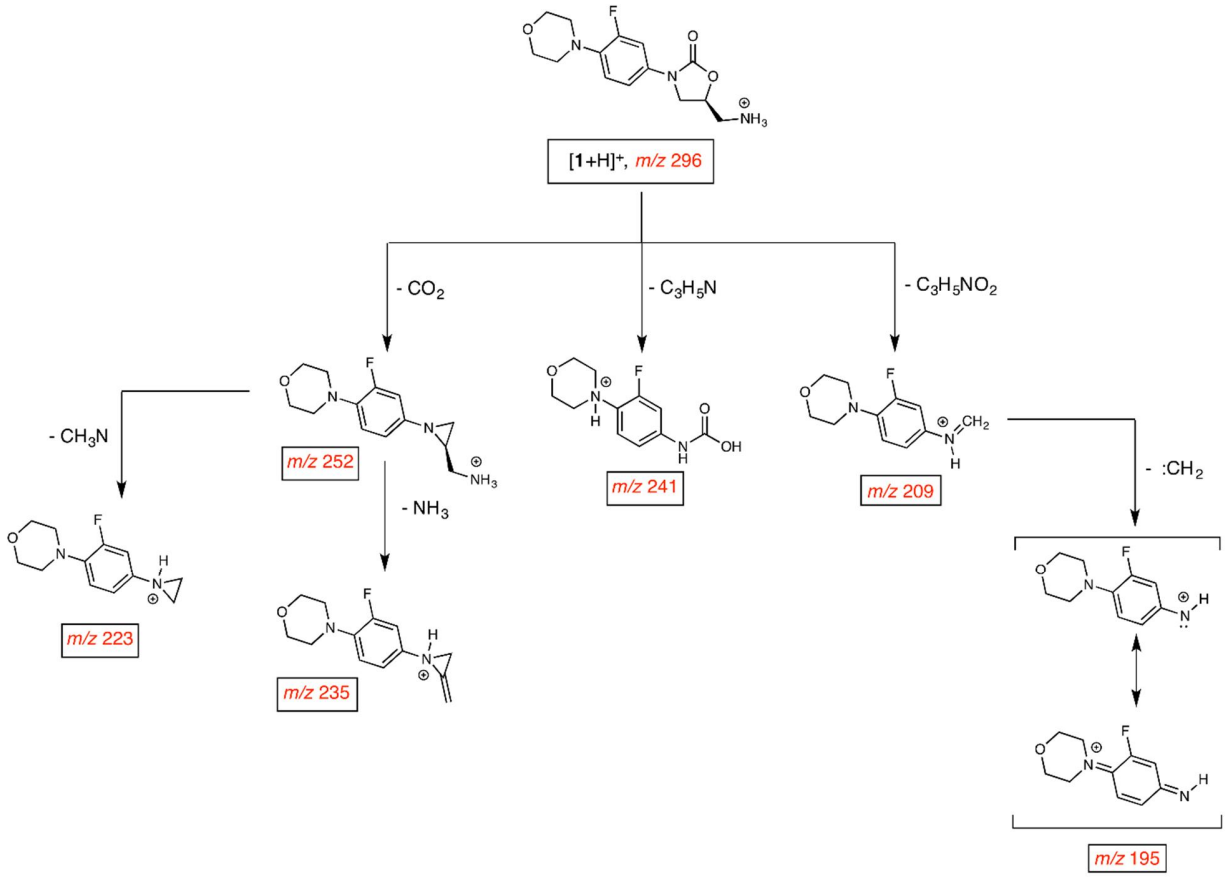
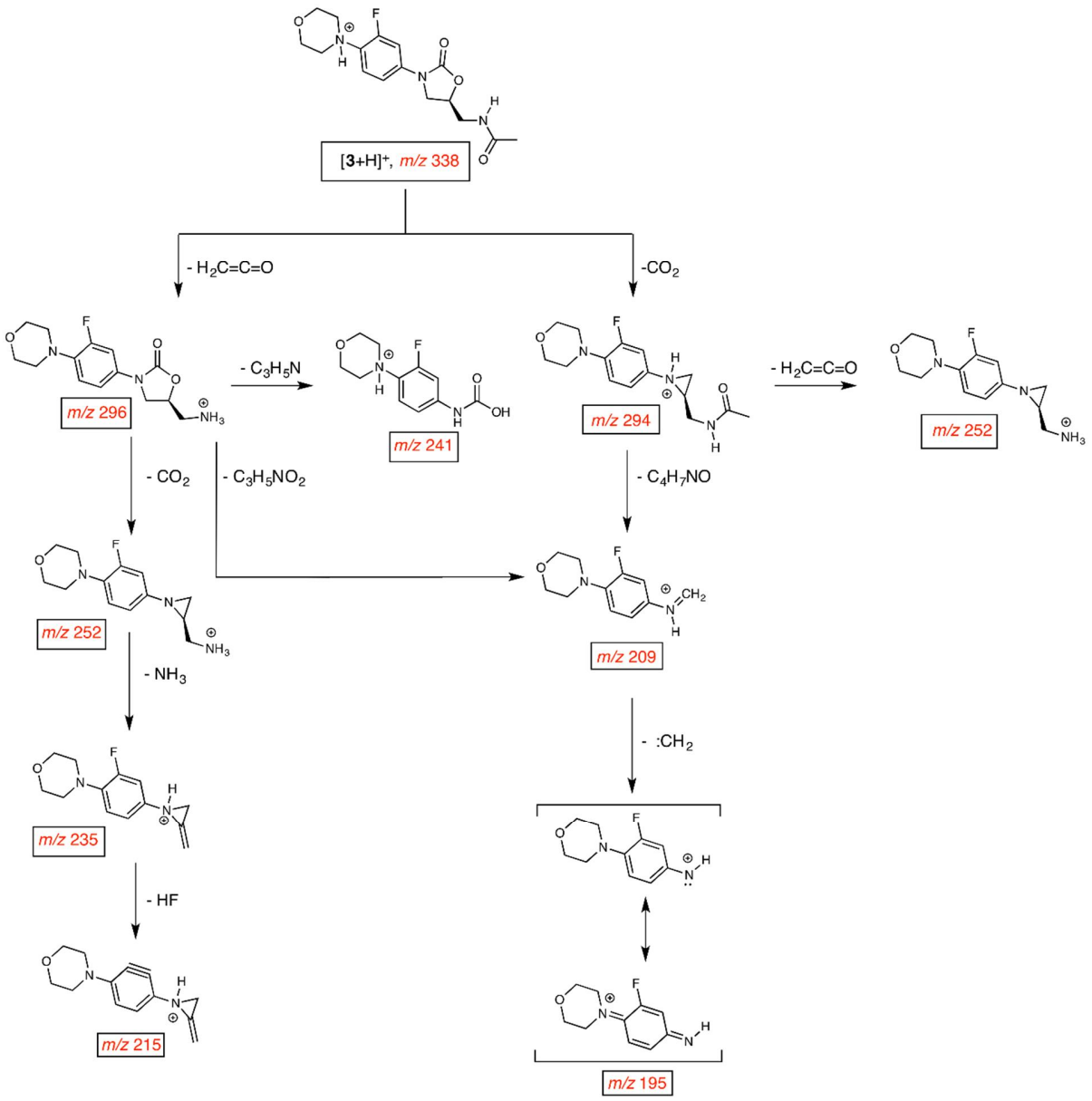


Figure S15. MS^3 mass spectra of the precursor ion *m/z* 296 (*m/z* 296 isolated from the product ion mass spectra of the precursor ion *m/z* 338) recorded with the ion trap mass spectrometer. (a) MS^3 mass spectrum recorded when the reaction was conducted online in the glass microreactor. The precursor ion *m/z* 338 is broken down with a fragmentation amplitude of 0.4 V, and subsequently isolated product ion *m/z* 296 is fragmented with a fragmentation amplitude of 0.35 V. (b) Reference MS^3 mass spectrum recorded offline, by directly infusing (flow rate 5 µL/min) a sample of 50 µM linezolid. The precursor ion *m/z* 338 is broken down with a fragmentation amplitude of 0.45 V, and subsequently isolated product ion *m/z* 296 is fragmented with a fragmentation amplitude of 0.4 V.



Scheme S2. Proposed fragmentation routes of the protonated precursor (5*S*)-5-(aminomethyl)-3-[3-fluoro-4-(4-morpholinyl)phenyl]-1,3-oxazolidin-2-one, *m/z* 296.



Scheme S3. Proposed fragmentation routes of the protonated final product linezolid, *m/z* 338.

Electrospray ionization (ESI) mass spectra measured with the direct infusion device

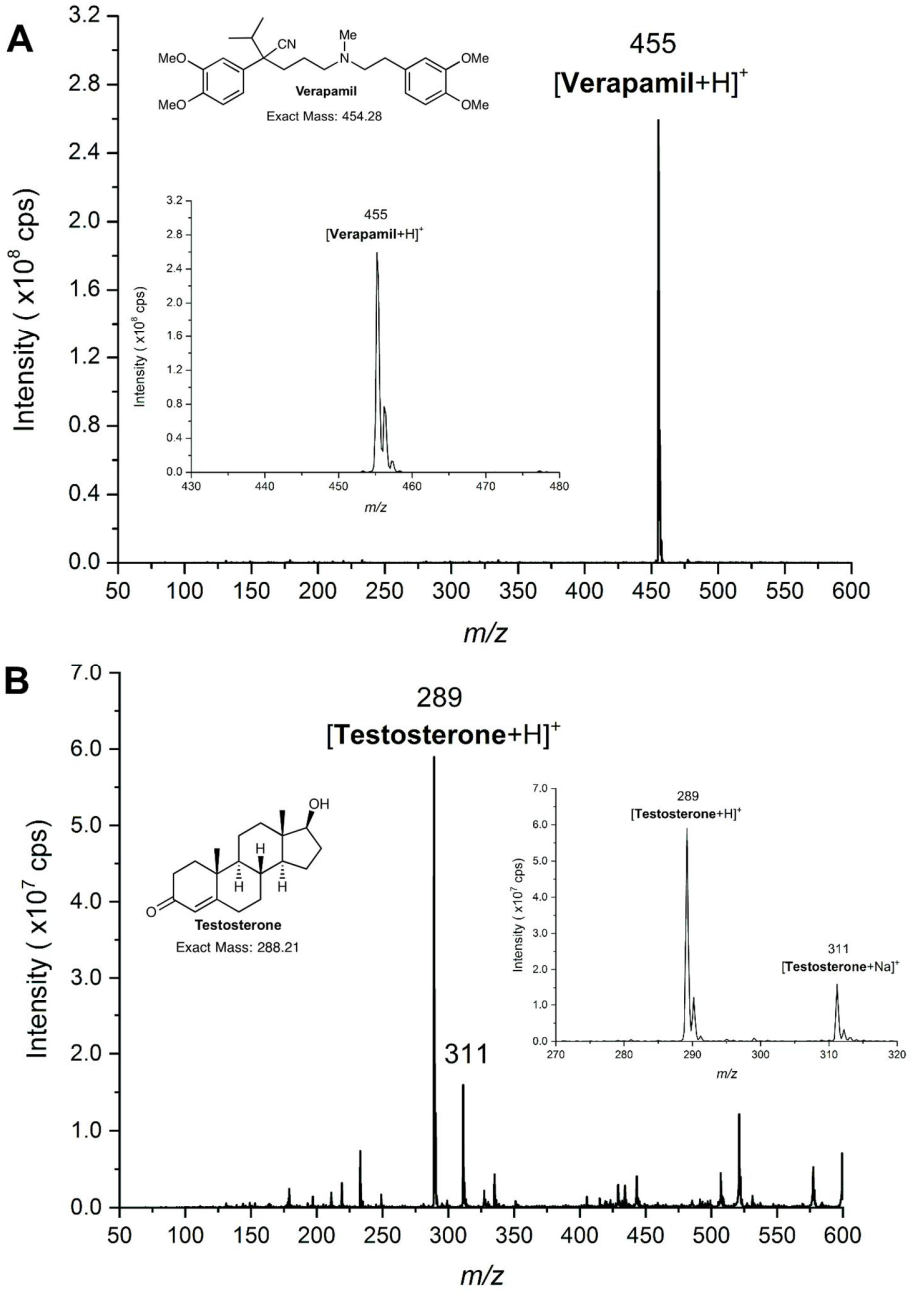


Figure S16. ESI mass spectra. An ESI mass spectrum averaged over 1 min when analyzing a sample of (a) 20 mM verapamil, and (b) 35 mM testosterone in methanol:water 80:20 + 1 vol% formic acid with the direct infusion glass device (45 mm channel).

Validation of the purity of (5*S*)-5-(aminomethyl)-3-[3-fluoro-4-(4-morpholinyl)phenyl]-1,3-oxazolidin-2-one and linezolid (commercial reference compound)

The purity of (5*S*)-5-(aminomethyl)-3-[3-fluoro-4-(4-morpholinyl)phenyl]-1,3-oxazolidin-2-one ($C_{14}H_{18}FN_3O_3$, molecular weight 295.31) and commercial linezolid ($C_{16}H_{20}FN_3O_4$, molecular weight 337.35) was confirmed using LC-MS and ^{1}H NMR (only the starting material). Based on the measurements, the commercially acquired compounds were pure.

LC-MS analysis for the validation

The mass spectrometer used in these measurements was a Waters Synapt G2 mass spectrometer (Waters Corporation, Massachusetts, USA) equipped with a Waters Acquity UPLC system. The separation column was an ACQUITY UPLC® BEH C-18 (ID 2.1 mm, length 50 mm, 1.7 µm particle size). Eluents; A: 100% water purified with a Milli-Q Plus purification system from Millipore (Molsheim, France) with 0.1 vol% formic acid and B: 100% Chromasolv®-grade acetonitrile with 0.1 vol% formic acid. When analyzing the (5*S*)-5-(aminomethyl)-3-[3-fluoro-4-(4-morpholinyl)phenyl]-1,3-oxazolidin-2-one sample, the gradient was the following: initially 98% of A and 2% of B, the portion of B was 40% at 5 min, the portion of B was 95% at 9 min, and decreased to 5% of B at 9.01 min and kept at this level up to 10 min (total runtime was 10 min). When the linezolid sample was analyzed, the gradient was the following: initially 95% of A and 5% of B, the proportion of B was 95% at 8 min, B was kept at 95% for 1 min, and decreased to 5% of B at 9.01 min and kept at this level up to 10 min (a total runtime of 10 min). The eluent flow rate was 0.6 mL/min and the column temperature was 40 °C. One-microliter samples of 30 mM (5*S*)-5-(aminomethyl)-3-[3-fluoro-4-(4-morpholinyl)phenyl]-1,3-oxazolidin-2-one (**1**) and 30 mM linezolid (**2**) in Chromasolv®-grade acetonitrile were injected to the UPLC. The mass spectrometer was operated in positive ESI mode with the following parameters: The capillary voltage was 3 kV, the ion source temperature 120 °C, and the desolvation temperature 360 °C. The measured mass range was 100-900 *m/z* with scan time 100 ms. Nitrogen was used as the cone gas, as well as desolvation gas. The cone gas flow rate was 10 L/h, and the desolvation gas flow rate was 800 L/h. The software MassLynx 4.0 (Waters Corporation, Massachusetts, USA) was used to process the obtained data.

The calculated exact mass for the protonated (5*S*)-5-(aminomethyl)-3-[3-fluoro-4-(4-morpholinyl)phenyl]-1,3-oxazolidin-2-one, $[M+H]^+$ was 296.1405 (calculated using "MolE - Molecular Mass Calculator v2.02" program from http://mods.rna.albany.edu/masspec/MoIE), and the measured mass for the $[M+H]^+$ peak was 296.14. The calculated exact mass for the protonated linezolid, $[M+H]^+$ was 338.1510 (calculated using "MolE - Molecular Mass Calculator v2.02" program from http://mods.rna.albany.edu/masspec/MoIE), and the measured mass for the $[M+H]^+$ peak was 338.15.

^{1}H NMR analysis for validation

A 110 mM solution of (5*S*)-5-(aminomethyl)-3-[3-fluoro-4-(4-morpholinyl)phenyl]-1,3-oxazolidin-2-one (**1**) was prepared in DMSO-d_6 (0.75 mL). The sample was transferred to an NMR tube made of borosilicate glass and analyzed with a Bruker Avance III 400 MHz NMR spectrometer (Bruker UK Limited, Coventry, UK). The frequency 400 MHz was used for acquiring the ^{1}H NMR spectrum. The chemical shifts (δ) are reported in parts per million (ppm), and the coupling constants are given in Hertz (Hz). The scale of the obtained NMR spectrum was adjusted according to the shift of DMSO-d_6 (2.5 ppm). The software MestReNova, version 11.0.3-18688 (Mestrelab Research S.L., Bajo, Spain) was used for processing the NMR data.

^{1}H NMR (400 MHz, DMSO-d_6) for (5*S*)-5-(aminomethyl)-3-[3-fluoro-4-(4-morpholinyl)phenyl]-1,3-oxazolidin-2-one (Figure S17): δ 7.51 (d, J = 15.1 Hz, 1H), 7.21 (dd, J = 8.8, 2.7 Hz, 1H), 7.06 (t, J = 9.0 Hz, 1H), 4.65–4.53 (m, 1H), 4.02 (t, J = 8.9 Hz, 1H), 3.82 (t, J = 6.8 Hz, 1H), 3.72 (d, J = 9.3 Hz, 4H), 2.95 (d, J = 9.3 Hz, 4H), 2.88–2.73 (m, 2H), 1.63 (s, 2H).

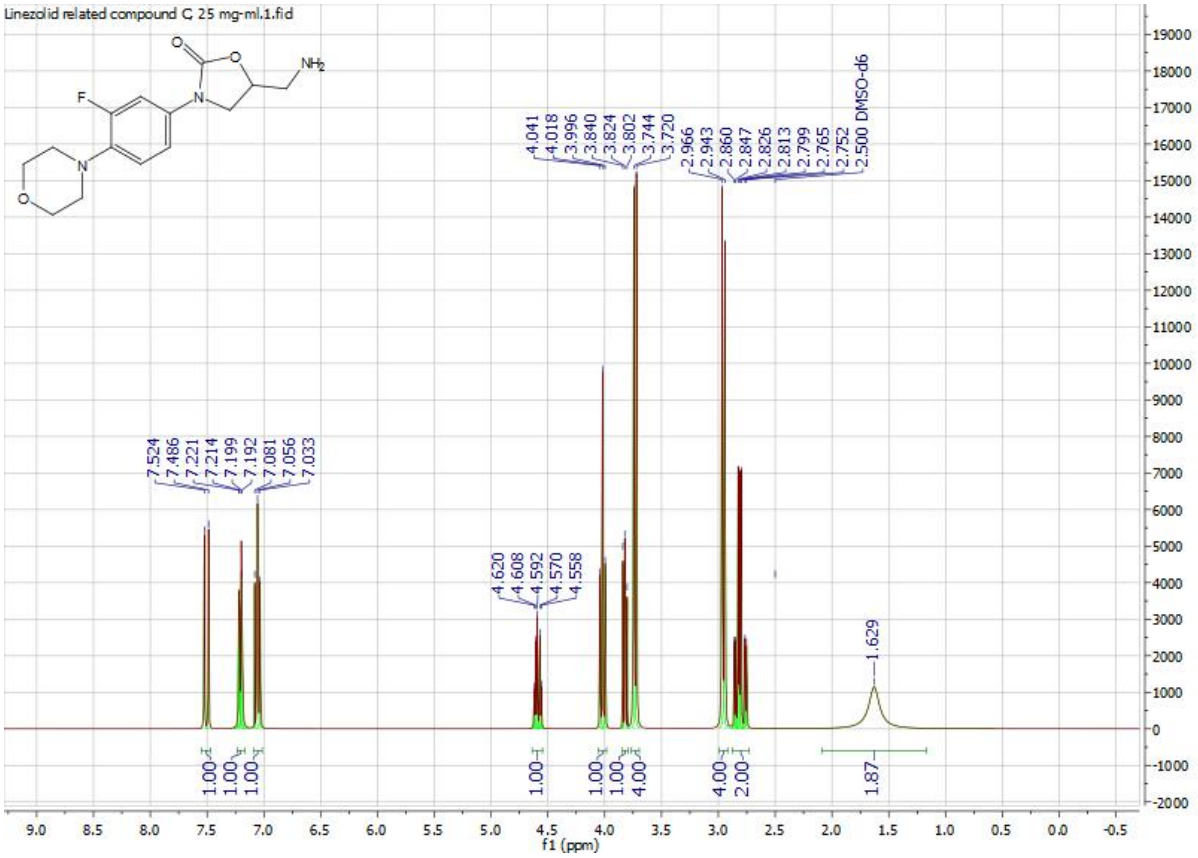


Figure S17. ^{1}H NMR spectrum of (5*S*)-5-(aminomethyl)-3-[3-fluoro-4-(4-morpholinyl)phenyl]-1,3-oxazolidin-2-one (**1**) in DMSO-d_6.

Movies

The movies S1 and S2 show testing of the integrity to the spiral device (Figure S9) by pumping air through it when the device was submerged under water. The movies reveal air bubbles emerging from the exit as well as from the inlet side. The original movies have been compressed.

Movie S1. Spiral device testing with air flow.
Movie S2. Spiral device testing with air flow, outlet only.

Reference

1. R. N. Tiwari and C.G. Bonde, *J. Liq. Chromatogr. Relat. Technol.* 2012, **35**, 188-203.