A Multi-Gram-Scale Stereoselective Synthesis of Z-Endoxifen

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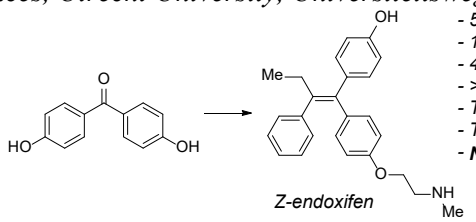

- *5 steps*
- *18% overall yield*
- *470 mmol scale synthesis delivered 37 g*
- *>97% purity, >99/1 Z/E*
- *Two columns chromatographies*
- *Two triturations*
- ***No RP-HPLC!***

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Experimental Section

General

Reagents and solvents for the initial and optimized synthesis

Column chromatography was carried out using either silica gel (40-63 µm, ScreeningDevices b.v.) or neutral alumina (activated, neutral, Brockmann Activity I, Sigma-Aldrich). Dihydroxybenzophenone, trimethylacetyl chloride, propiophenone, zinc dust, titanium tetrachloride were all purchased from Sigma-Aldrich and used without further purification. Sodium hydroxide was purchased from Merck. Ethyl acetate, dichloromethane, hexanes, and methanol were all purchased from Biosolve B.V. For the synthesis of alcohol **4**: 2-(methylamino)ethanol (Sigma-Aldrich), ethyl chloroformate, trimethylamine (all Sigma-Aldrich), dichloromethane. THF (both Biosolve B.V.). All deuterated solvents for NMR analysis were purchased from Cambridge Isotope Laboratories Inc. and used without further treatment. Compounds **3**, **5**, **6** & *Z*-endoxifen were synthetized under reduced light under the fumehood and stored in the dark at -30 °C. H_2O refers to Millipore grade distilled water.

Reagents and solvents for the scaled-up synthesis

All reagents and solvents were obtained from commercial sources (Sigma Aldrich, Acros, Fluorochem and Combi-Blocks) and were used without further purification unless otherwise specified. All deuterated solvents for NMR analysis were purchased from Acros. Reverse-phase liquid chromatography-mass spectrometry (LC-MS) analysis of *Z*-endoxifen reported in Figure S1 was performed on an Applied Biosystems Single Quadrupole Electrospray Ionization Mass Spectrometer API-150EX in positive mode using a Jupiter C4-column 150 x 2.0 mm. Eluent conditions (CH_3CN/H_2O/1% formic acid): 0-2 min, isocratic, 5 % CH_3CN; 2-10 min, linear gradient, 5 – 70 %; 10-12 min, isocratic, 70 %; 12-15 min, linear gradient, 70 – 5 %. CH_3CN refers to HPLC grade acetonitrile purchased from Biosolve B.V. The formic acid used was ULC-MS grade, 99% purchased from Biosolve B.V. H_2O refers to MilliQ Ultrapure water for UHPLC and LC-MS. LC-UV-MS analysis of *Z*-endoxifen reported in Figure S16 was performed using a Shimadzu LC-MS with Phenomenex Luna 5u C18(2) 100A (100 x 4.6 mm) column with PDA Detection. ^{1}H-NMR measurements made on Z-endoxifen during the initial synthesis work (Figures S2-S4) were performed on a 400 MHz NMR (Varian Mercury). Proton chemical shifts in 1D ^{1}H-NMR spectra are reported in ppm and calibrated against either the tetramethylsilane or residual $CHCl_3$ (s, 7.26 ppm), *d5*-DMSO (quintet, J_{HD} = 1.9 Hz), as the internal standard. Carbon chemical shifts in the 1D ^{13}C-NMR spectra are reported in ppm and calibrated against the $CDCl_3$ (77.16 ppm, t, J_{CD} = 32 Hz) or *d6*-DMSO signals (39.52 ppm, septet, J_{CD} = 21 Hz). ^{1}H-NMR, ^{13}C-NMR and ^{19}F NMR measurements made during the optimized synthesis of *Z*-endoxifen (Figures S6-S15) were recorded on a Varian VNMRS: 7.05 Tesla magnet from Oxford Instruments, indirect detection probe 300 MHz ^{1}H{^{15}N-^{31}P}, Direct drive console including PFG module and a Varian MP300: 7.05 Tesla magnet from Oxford Instruments, 4 nuclei autoswitchable probe ^{1}H/^{19}F/{^{15}N-^{31}P}, Mercury plus console. The splitting patterns are designated as: s, singlet; d, doublet; t, triplet; q, quartet; br s, broad singlet; m, multiplet. The purity-determination by qNMR with 3,5-dinitrobenzoic acid as reference were calculated by comparing the combined integrals of the aromatic signals of 3,5-dinitrobenzoic acid and the combined integral of the signals at δ 4.08, 3.25 and 0.83 ppm of

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Z-Endoxifen. For the purity-determination by qNMR with maleic acid the integral of the signal at δ 6.16 ppm was compared with the integral of the combined signals at δ 4.05 and 0.83 ppm.

Determination of *E/Z* ratios of endoxifen by HPLC analysis described in Figures S5

Measurement were performed according to the methods described in Teunissen et al., 2011.[1]

Reagents and chemicals

Acetonitrile and methanol were obtained from Biosolve Ltd. (Amsterdam, the Netherlands). Ammonium formate was purchased from Acros Organics (Geel, Belgium). Formic acid and LiChrosolv water for HPLC were purchased from Merck (Darmstadt, Germany).

Instrumentation & gradient conditions

An Agilent HPLC system was used consisting of an 1100 series binary pump, column oven, on-line degasser and autosampler (Agilent Technologies, Palo Alto, CA, USA). Mobile phase A was prepared by adjusting a 4.0 mM ammonium formate solution to pH 3.5 with a 98% formic acid solution. Mobile phase B consisted of 100% acetonitrile. Mobile phases A and B were pumped through a Kinetex C18 100 Å column (150 x 2.1 mm I.D., 2.6 µm; Phenomenex) at a flow rate of 0.4 mL/min using a gradient as shown in Table 2. The analytical column was protected by a KrudKatcher inline filter (Phenomenex, Torrance, CA, USA). The separation was performed at 60˚C. Volumes of 15 µL were injected using the autosampler thermostatted at 7 ˚C. The column was equilibrated for 3 minutes before the next injection, leading to a total run time of 10 minutes. The autosampler needle was rinsed with acetonitrile before and after each injection. During the first and last 1.0 minute the eluate was directed to waste using a divert valve to prevent the introduction of endogenous compounds into the mass spectrometer. The HPLC gradient conditions used to separate *Z*- and *E*-isomers of endoxifen using the above conditions are as follows: under a constant flow rate 0.40 mL/min, mobile phase A = 4.0 mM ammonium formate buffer pH 3.5, mobile phase B = acetonitrile; t = 0.00 min, A = 70%, B = 30%; t = 6.00 min, 47.5/52.5; t = 6.01 min, 20/80; t = 7.00 min, 20/80; t = 7.01 min, 70/30; t = 10.00 min, 70/30.

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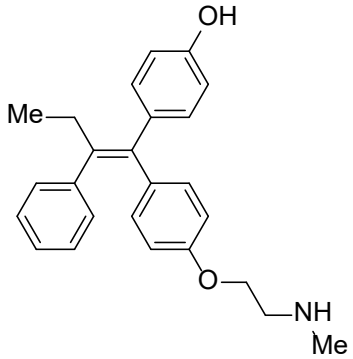
Chemical synthesis of *Z*-endoxifen



Initial synthesis

The initial synthesis of *Z*-endoxifen was performed according to the synthetic route depicted in Figure 2 of the main manuscript and the synthetic protocols described in previous work published by Gauthier & Labrie et al.[2] and Fauq et al.[3] The crude material was purified by silica gel column chromatography ($MeOH/CH_2Cl_2$) to afford *Z*-endoxifen (Figure S1) as a 3.6/1 *E/Z* mixture determined by ^{1}H-NMR (Figures S2 & S3) and HPLC (Figure S5, left panel).

Optimized synthesis

For the optimized synthesis, the crude material was instead purified by column chromatography using neutral alumina ($MeOH/CH_2Cl_2$) to afford *Z*-endoxifen as a 96/4 *Z/E* mixture determined by ^{1}H-NMR (Figure S4) and HPLC (Figure S5, right panel)

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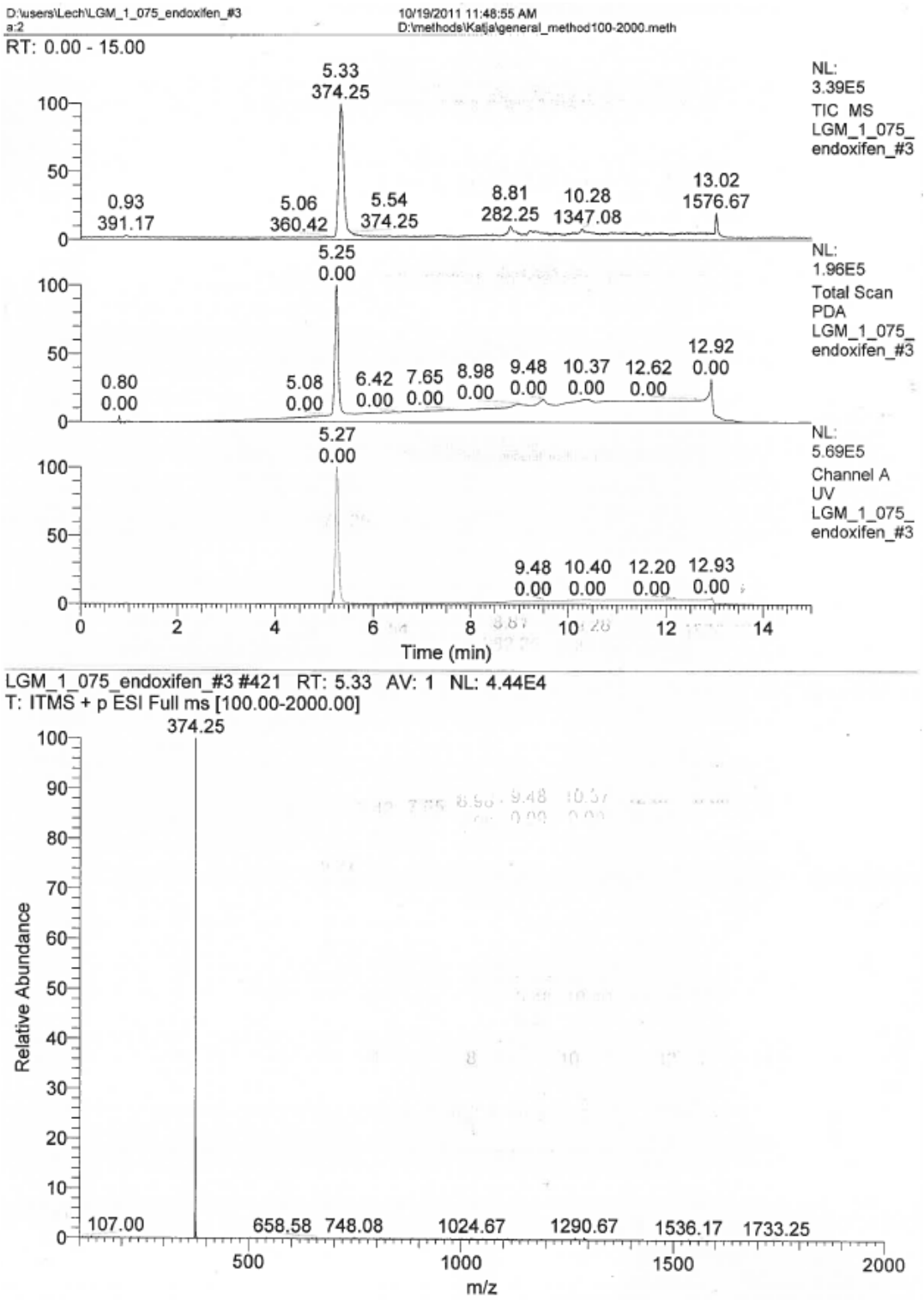


Figure S1. LC-MS analysis of *Z*-endoxifen after purification by silica gel column chromatography ($MeOH/CH_2Cl_2$). Expected 374.21, observed 374.25 $[M+H]^+$.

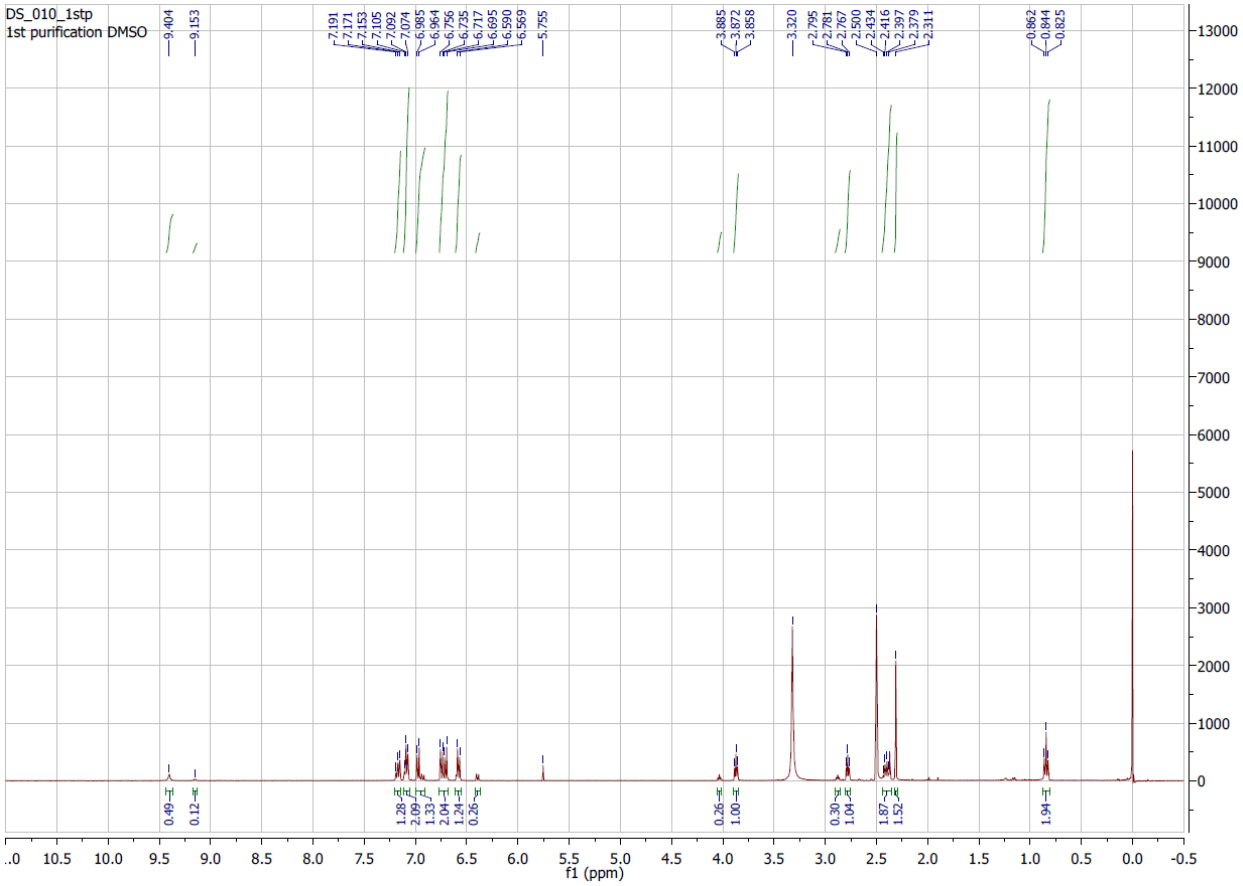


Figure S2. 1D ^{1}H NMR (*d6*-DMSO, 400 MHz) of *Z*-endoxifen after purification by silica gel column chromatography ($MeOH/CH_2Cl_2$). *Z/E* ratio = 3.6/1 (see Figure S5)

Comparison of experimental and literature 1D ^{1}H NMR shift values for Z-endoxifen

Literature [S. M. Ali, et al. *Bioorg. Med. Chem. Lett.*, *2010*, **20**, 2665]:[4] ^{1}H-NMR (400 MHz, *d6*-DMSO): δ 0.85 (t, *J* = 7.24 Hz, 3H), 2.29 (s, 3H), 2.42 (q, *J* = 7.2 Hz, 2 H), 2.74 (t, *J* = 5.59 Hz, 3H), 3.86 (t, *J* = 5.56 Hz, 2H), 6.58 (d, *J* = 8.78 Hz, 2H), 6.71 (d, *J* = 8.56 Hz, 2H), 6.75 (d, *J* = 8.68 Hz, 2H), 6.98 (d, *J* = 8.2 Hz, 2H), 7.08–7.13 (m, 3H), 7.15–7.19 (m, 2H), 9.38 (br s, 1H)

This publication (only values for major isomer, Z-isomer are reported) [internal code: DS_010_1stp]: ^{1}H-NMR (400 MHz, *d6*-DMSO): δ 0.84 (t, *J* = 7.2 Hz, 3H), 2.31 (s, 3H), 2.40 (q, *J* = 7.2 Hz, 2 H), 2.78 (t, *J* = 5.6 Hz, 3H), 3.87 (t, *J* = 5.6 Hz, 2H), 6.56 (d, *J* = 8.4 Hz, 2H), 6.69 (d, *J* = 8.8 Hz, 2H), 6.75 (d, *J* = 8.4 Hz, 2H), 6.98 (d, *J* = 8.4 Hz, 2H), 7.07–7.11 (m, 3H), 7.15–7.19 (m, 2H), 9.40 (br s, 1H).

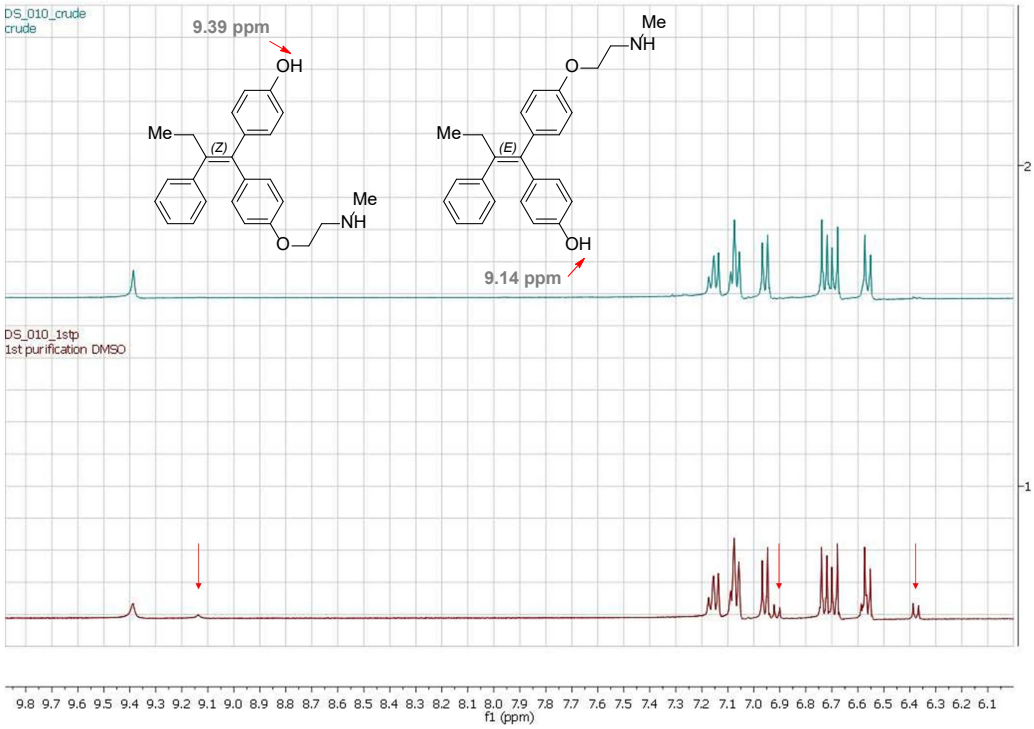


Figure S3. A portion of the 1D ^{1}H NMR spectrum (*d6*-DMSO, 400 MHz) of *Z*-endoxifen shown in Figure 2 after aqueous work-up but before (crude, top) and then after purification (bottom) by silica gel chromatography. *Z/E* ratio = 3.6/1 (see Figure S5)

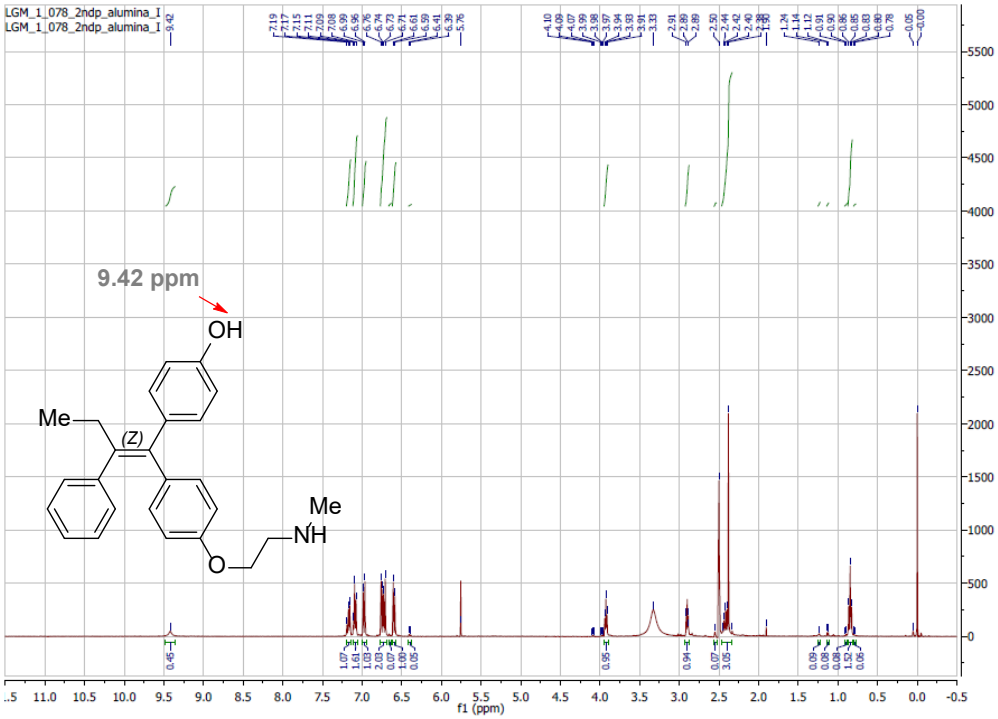


Figure S4. 1D 1H NMR (*d6*-DMSO, 400 MHz) of *Z*-endoxifen after purification using neutral alumina. *Z/E* ratio = 96/4 (see Figure S5)

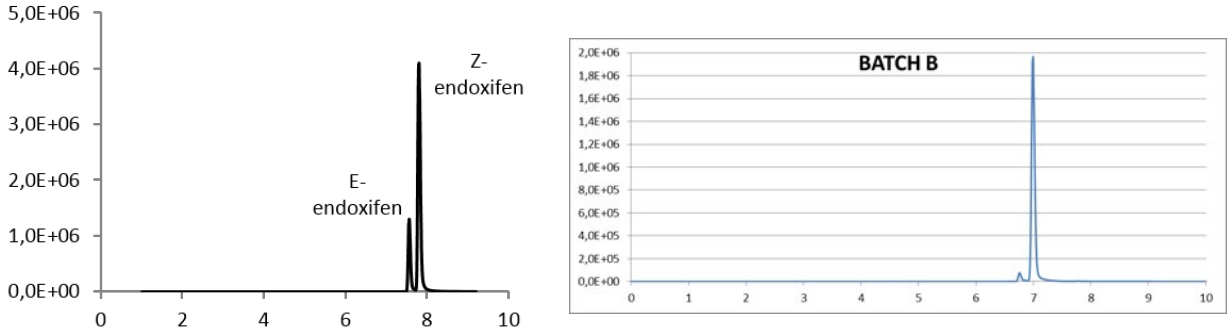
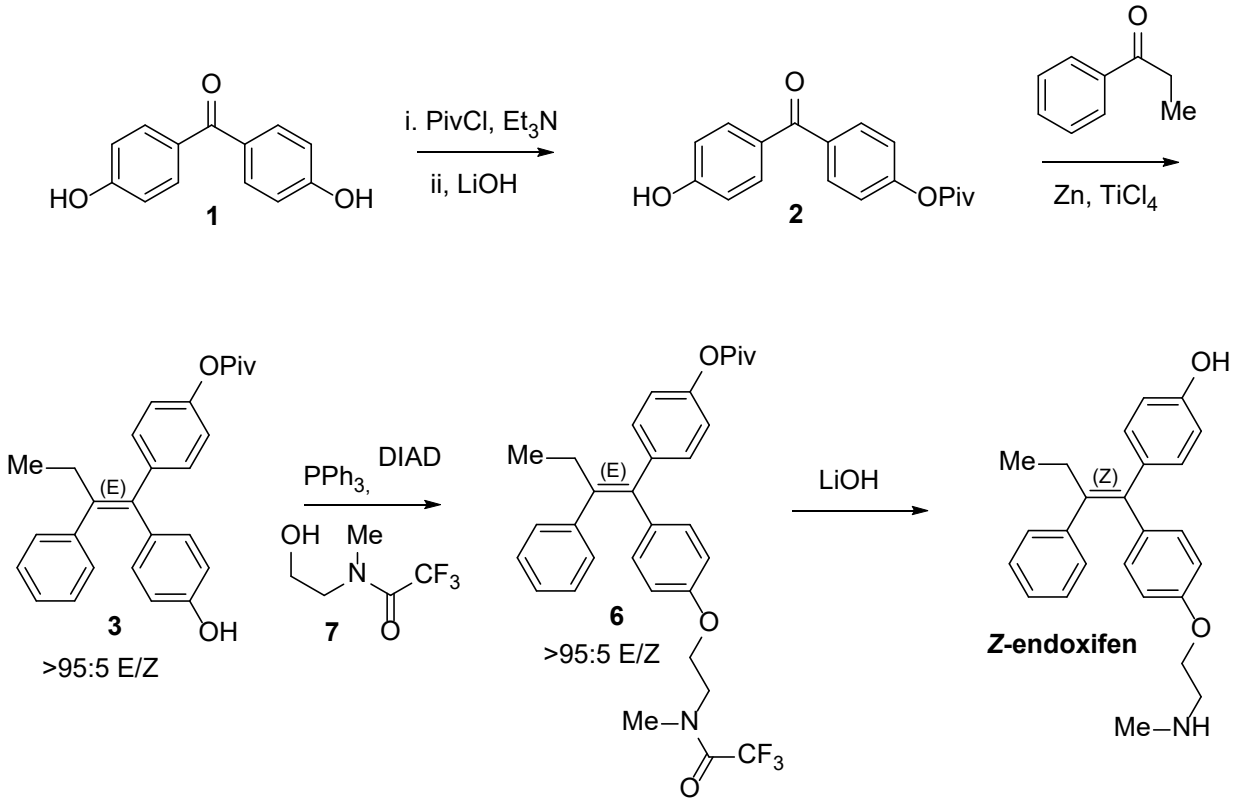


Figure S5. Comparison of LC-UV data for *Z*-endoxifen analyzed after purification on silica gel chromatography (left) – Z/*E* ratio = 3.6/1 – and neutral alumina (right) – *Z/E* ratio = 96/4.

Large-scale synthesis of *Z*-endoxifen



Scheme S1. Overview of large-scale synthetic route to *Z*-endoxifen.(see Figure 3 of main manuscript)

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4-(4-hydroxybenzoyl)phenyl pivalate 2

Bis(4-hydroxyphenyl)methanone **1** (100 g, 0.47 mol) was dissolved in 1 L of THF and cooled to 0 °C. Triethylamine (188.7 g, 1.87 mol) was added followed by the dropwise addition of PivCl (141.7 g, 1.17 mol). More THF (250 mL) was added and the mixture was stirred overnight. The mixture was quenched with 500 mL of water and extracted with ethyl acetate (2x). The combined organic layers were washed with 1N HCl (500 mL) and brine and dried over Na_2SO_4. Concentration afforded 194.3 g of the crude bis protected benzophenone. The material was dissolved in 750 mL of THF and 50 mL of methanol. $LiOH.H_2O$ (21.4 g, 0.5 mol) was added and the mixture was stirred for 1 hour. More $LiOH.H_2O$ (5 g, 0.12 mol) was added and the mixture was stirred for an additional 30 min. The mixture was concentrated. At this point a second equal batch was run and combined with the first one. The combined batches were coated on silica and purified by means of column chromatography (silica; CH_2Cl_2/EtOAc 20:1→ CH_2Cl_2/THF 20:1) affording 4-(4-hydroxybenzoyl)phenyl pivalate as a white solid (140 g, 47%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.38 (s, 9H) 6.41 (br s, 1H), 6.90 (d, J = 9 Hz, 2H), 7.18 (d, J = 8,7 Hz, 2H), 7.75 (d, J = 8,7 Hz, 2H), 7.80 (d, J = 8.7 Hz, 2H).

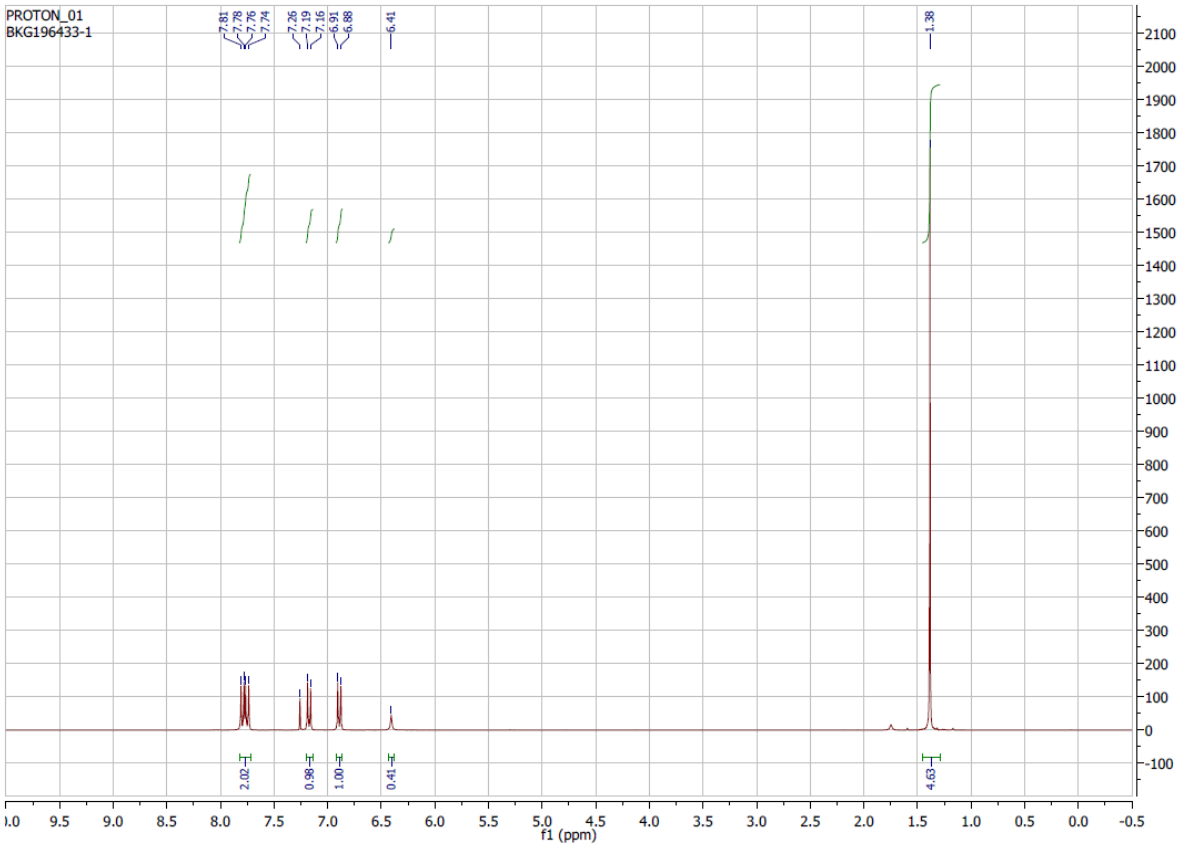


Figure S6. 1D ^{1}H NMR spectrum of pure **2** ($CDCl_3$, 300 MHz).

2,2,2-trifluoro-*N*-(2-hydroxyethyl)-*N*-methylacetamide 7

Ethyl trifluoroacetate (92.3 g, 0.65 mol) was slowly added to 2-(methylamino)ethanol (50 g, 0.67 mol) at 10 °C. The mixture was stirred for 3 hours at rt and CH_2Cl_2 was added. The mixture was washed with 1N HCl and brine and dried over Na_2SO_4. Evaporation of the solvent afforded 2,2,2-trifluoro-*N*-(2-hydroxyethyl)-*N*-methylacetamide as colorless oil (84 g, 75%, 2.7:1 mixture of rotamers). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.28 (br s, 1H), 3.10 (apparent s, 3H – minor rotamer), 3.22 (m, 3H – major rotamer), 3.56-3.62 (m, 2H) 3.81-3.86 (m, 2 H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 35.3, 36.2 (q, J = 3.8 Hz), 51.3 (q, J = 3.0 Hz), 51.9, 59.3, 59.8, 116.44 (q, J = 285 Hz), 116.41 (q, J = 285 Hz), 157,40 (q, J = 35.3 Hz), 157,55 (q, J = 35.3 Hz); ^{19}F-NMR (282 MHz) -69.9 (major rotamer), -68.2 (minor rotamer).

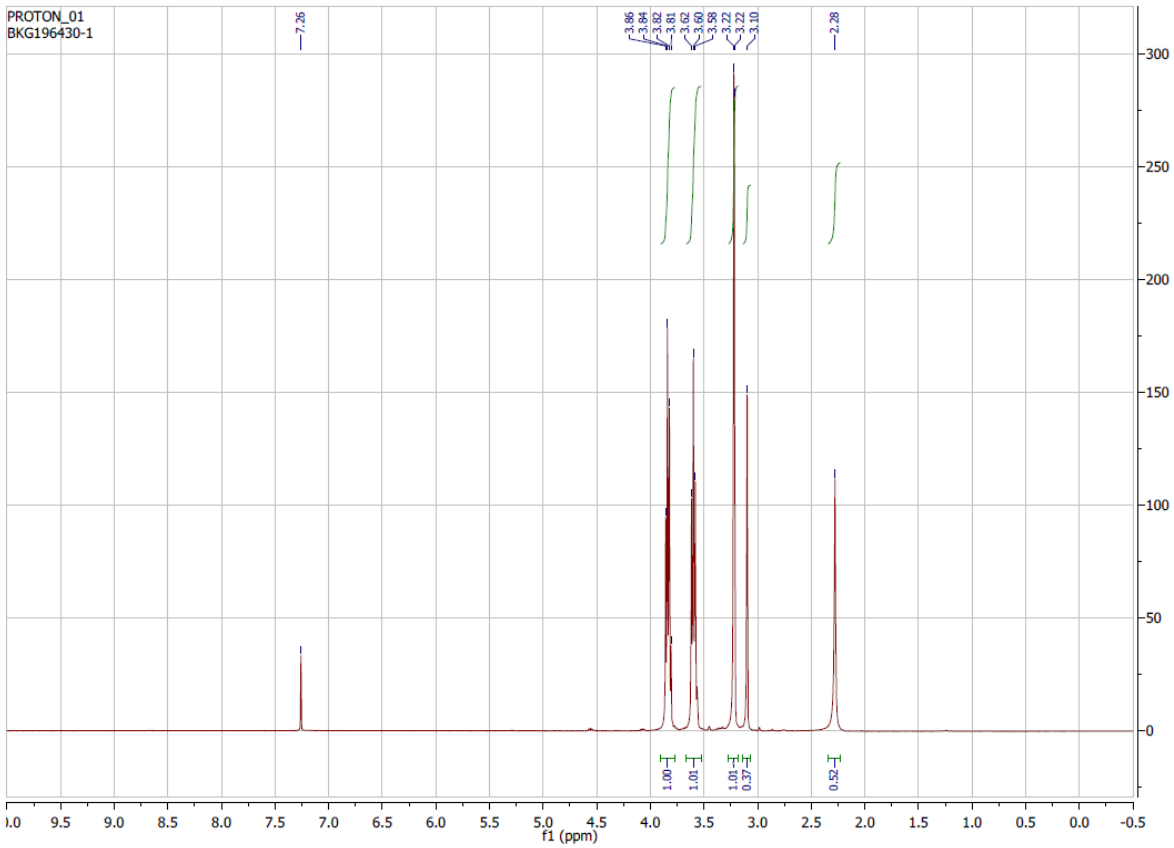


Figure S6. 1D ^{1}H NMR spectrum of pure **7** ($CDCl_3$, 300 MHz).

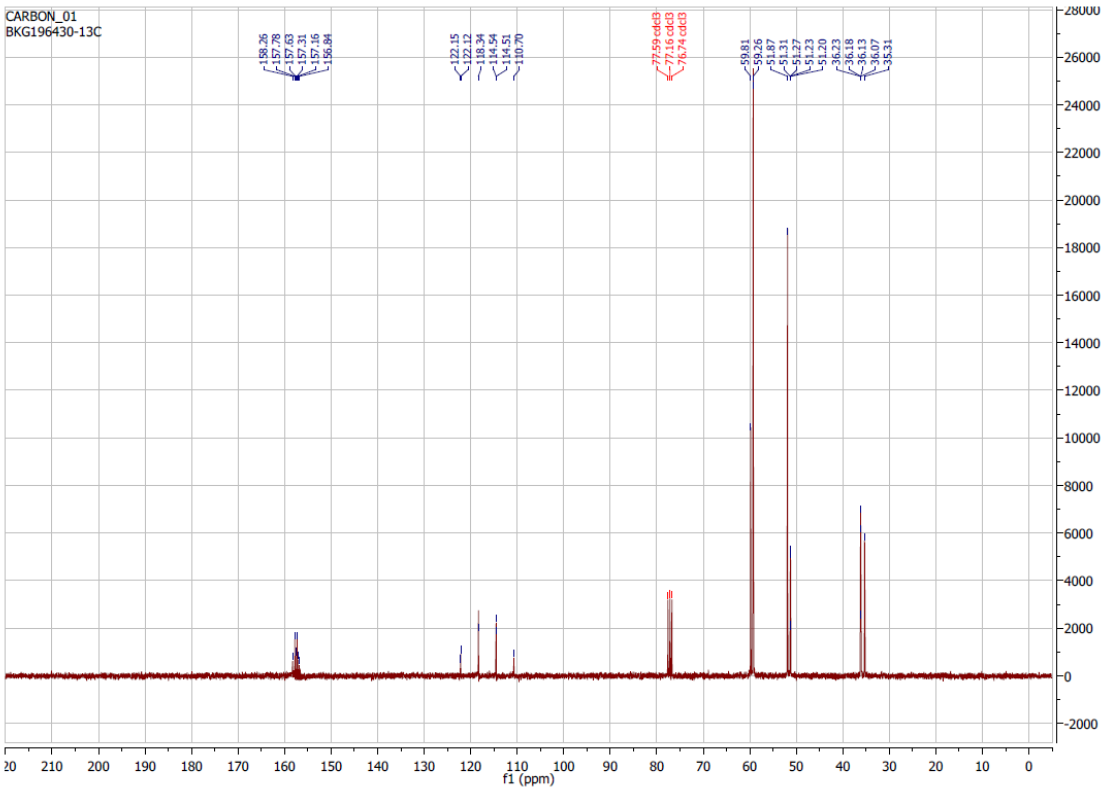


Figure S7. 1D ^{13}C NMR spectrum of pure **7** ($CDCl_3$, 75 MHz).

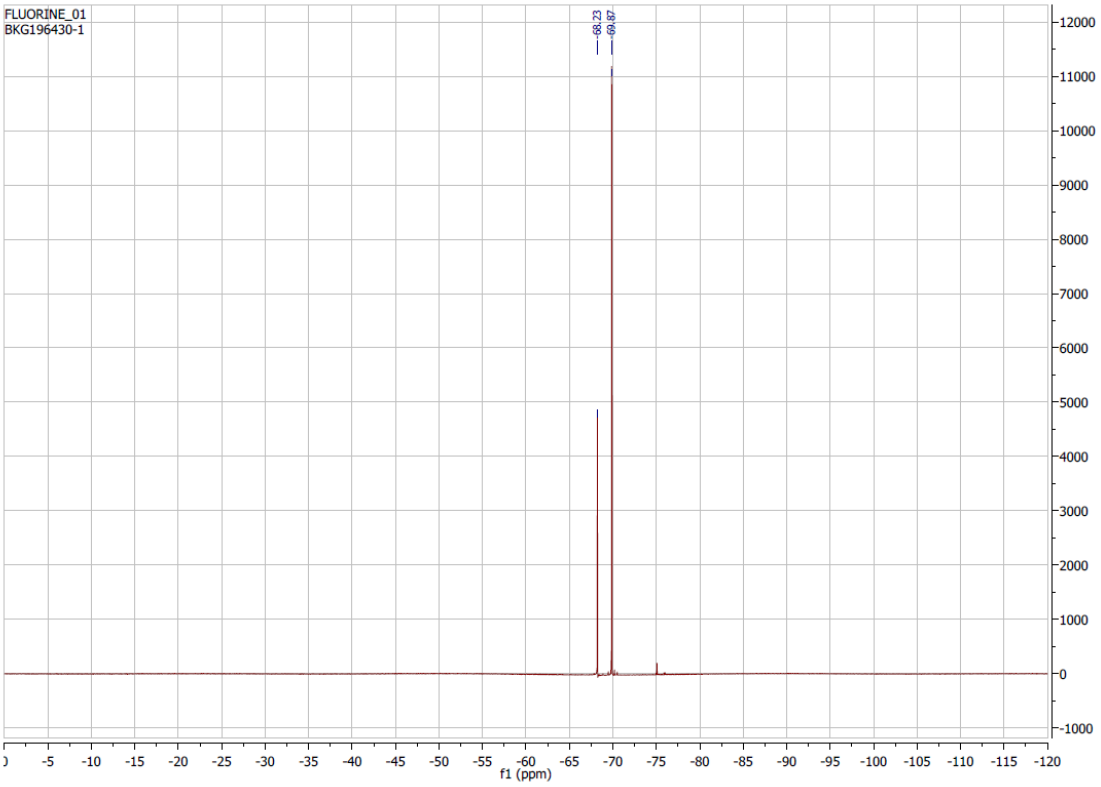


Figure S8. 1D ^{19}F NMR spectrum of pure **7** ($CDCl_3$, 282 MHz).

(*E*)-4-(1-(4-hydroxyphenyl)-2-phenylbut-1-en-1-yl)phenyl pivalate 3

To a suspension of zinc dust (153.4 g, 2360 mmol) in anhydrous THF (2 L) was added $TiCl_4$ (223.8 g, 1180 mmol) dropwise at 0-10 °C. The mixture was refluxed for 2 h and then cooled to 40 °C. A mixture of 4-(4-hydroxybenzoyl)phenyl pivalate (88 g, 295 mmol) and propiophenone (123.5 g, 922 mmol) in anhydrous THF (4 L) was added at once and the mixture was refluxed for 5 h. Upon completion, the reaction mixture was cooled to 0 °C and quenched with 10% K_2CO_3 (4 L). Celite was added and the mixture was stirred for 30 min before the organic layer was sucked from the mixture and filtered over Celite. The aqueous/celite mixture was stirred up with EtOAc and the organic layer was sucked from the mixture and filtered over Celite as before. This was repeated three times. The combined organic extracts were successively washed with 10% K_2CO_3 and brine, dried over $MgSO_4$, filtered, and the filtrate concentrated under reduced pressure to afford a crude (*E*)-4-(1-(4-hydroxyphenyl)-2-phenylbut-1-en-1-yl)phenyl pivalate (234 g). After thorough evaporation of the solvents the crude material was triturated in 200 mL of methanol (4x) affording pure (*E*)-4-(1-(4-hydroxyphenyl)-2-phenylbut-1-en-1-yl)phenyl pivalate as a white solid. The filtrate was concentrated and the obtained material was triturated in 200 mL of methanol (3x). Extra material was obtained and this was combined with the first crop. The material was dried in vacuo at 50 °C overnight to afford 65 g of pure and dry (*E*)-4-(1-(4-hydroxyphenyl)-2-phenylbut-1-en-1-yl)phenyl pivalate (55%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.91 (t, J = 7.2 Hz, 3H), 1.37 (s, 9H), 2.46 (q, J = 7.2 Hz, 2H), 4,50 (s, 1H), 6.47 (d, J = 8.4 Hz, 2H), 6.72 (d, J = 8,7 Hz, 2H), 7.03-7.25 (m, 9H).

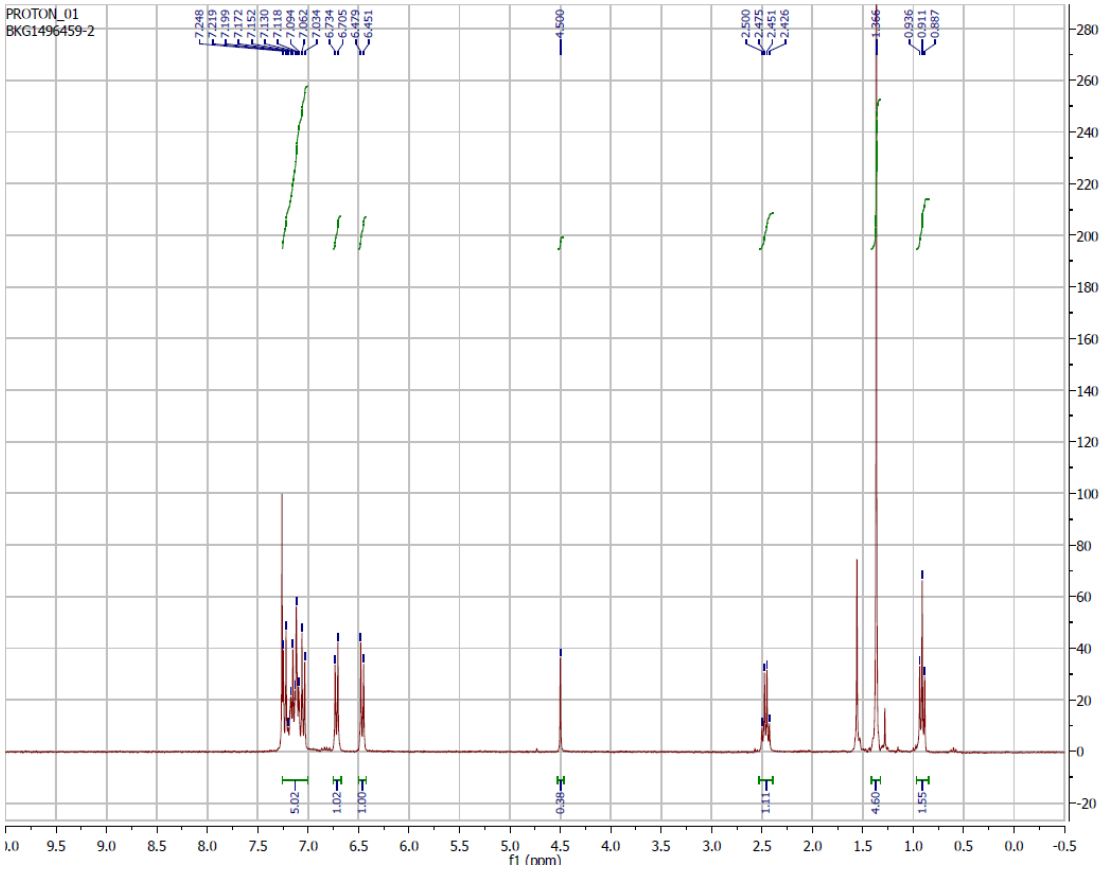


Figure S9. 1D ^{1}H NMR spectrum of pure **3** ($CDCl_3$, 300 MHz).

(*E*)-4-(2-phenyl-1-(4-(2-(2,2,2-trifluoro-*N*-methylacetamido)ethoxy)phenyl)but-1-en-1-yl)phenyl pivalate 6

(*E*)-4-(1-(4-hydroxyphenyl)-2-phenylbut-1-en-1-yl)phenyl pivalate (49.5 g, 123.8 mmol) was dissolved in 2 L of THF and PPh_3 (66.8 g, 255 mmol) was added. The mixture was cooled to 10°C and a mixture of DIAD (50.5 g, 250 mmol) and 2,2,2-trifluoro-*N*-(2-hydroxyethyl)-*N*-methylacetamide (42.8 g, 250 mmol) in 500 mL of THF was added drop wise over 5 hours. The mixture was stirred for an additional 18 hours. Since the reaction was not complete more PPh_3 (39.3 g, 150 mmol) was added to the mixture. Also a mixture of DIAD (30.3 g, 150 mmol) and 2,2,2-trifluoro-*N*-(2-hydroxyethyl)-*N*-methylacetamide (25.7 g, 150 mmol) in 300 mL of THF was added drop wise over 5 hours. The mixture was stirred overnight and subsequently concentrated in vacuo. Note that some stereorandomization of 6 is detected on aqueous workup, but not if the crude is purified by neutral alumina column chromatography (heptanes/EtOAc 10:1) immediately after evaporation of the reaction solvents under reduced pressure affording (*E*)-4-(2-phenyl-1-(4-(2-(2,2,2-trifluoro-*N*-methylacetamido)ethoxy)phenyl)but-1-en-1-yl)phenyl pivalate as a white solid (56.8 g, 83%, 2.6:1 mixture of rotamers).^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.91 (t, *J* = 6 Hz, 3H), 1.36 (s, 9H), 2.47 (q, *J* = 6 Hz, 2H), 3.11 (s, 3H – minor rotamer), 3.23 (s, 3H – major rotamer), 3.72-3.77 (m, 2H – mixture of rotamers), 3.99-4.07 (m, 2H – mixture of rotamers), 6.52 (d, *J* = 9 Hz, 2H), 6.77 (d, *J* = 9 Hz, 2H), 7.03-7.24 (m, 9H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 13.53, 27.14, 29.05, 36.05, 37.07, 39.10, 48.67, 49.50, 65.43, 65.98, 113.22, 116.35 (q, *J* = 286 Hz), 116.45 (q, *J* = 286 Hz), 121.12, 126.14, 127.91, 129.62, 130.38, 132.06, 135.93, 136.17, 137.16, 140.99, 141.98, 142.20, 149.72, 155.88, 156.13, 157,12 (q, *J* = 35.3 Hz), 157,14 (q, *J* = 36.0 Hz); ^{19}F-NMR (282 MHz) -69.9 (major rotamer), -68.3 (minor rotamer).

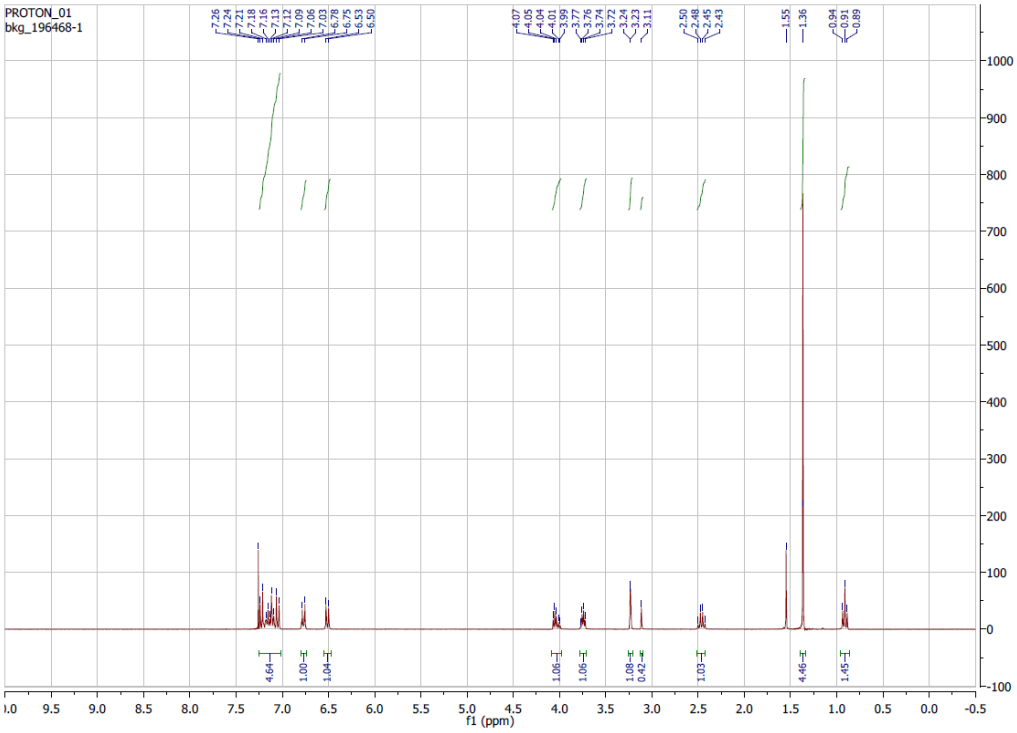


Figure S10. 1D ^{1}H NMR spectrum of pure **6** ($CDCl_3$, 300 MHz).

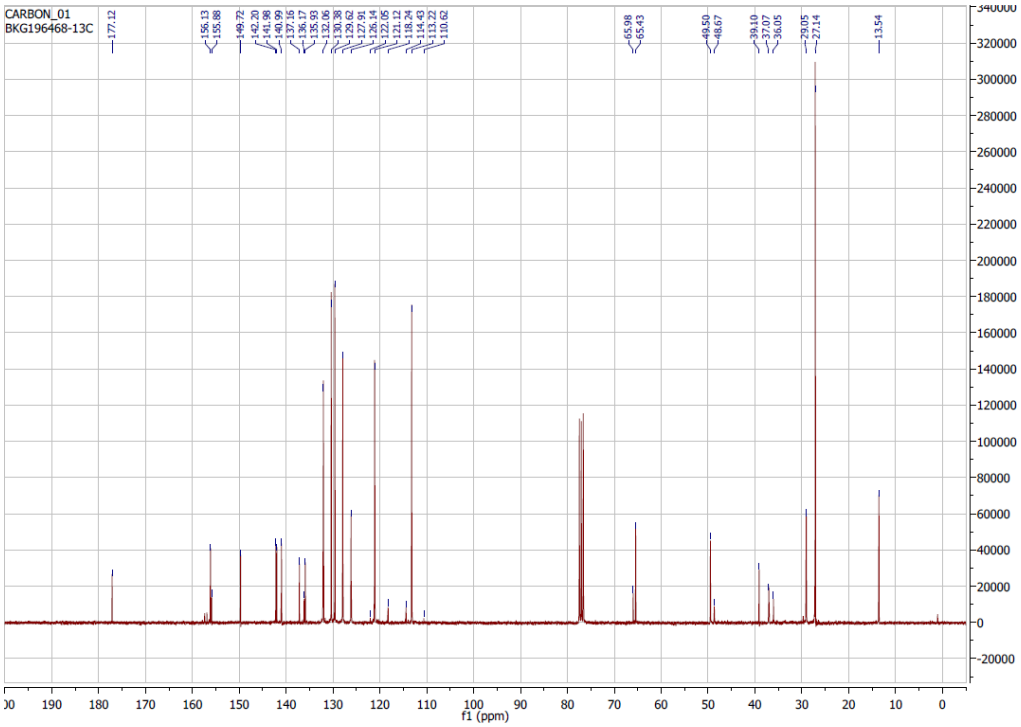


Figure S11. 1D ^{13}C NMR spectrum of pure **6** ($CDCl_3$, 75 MHz).

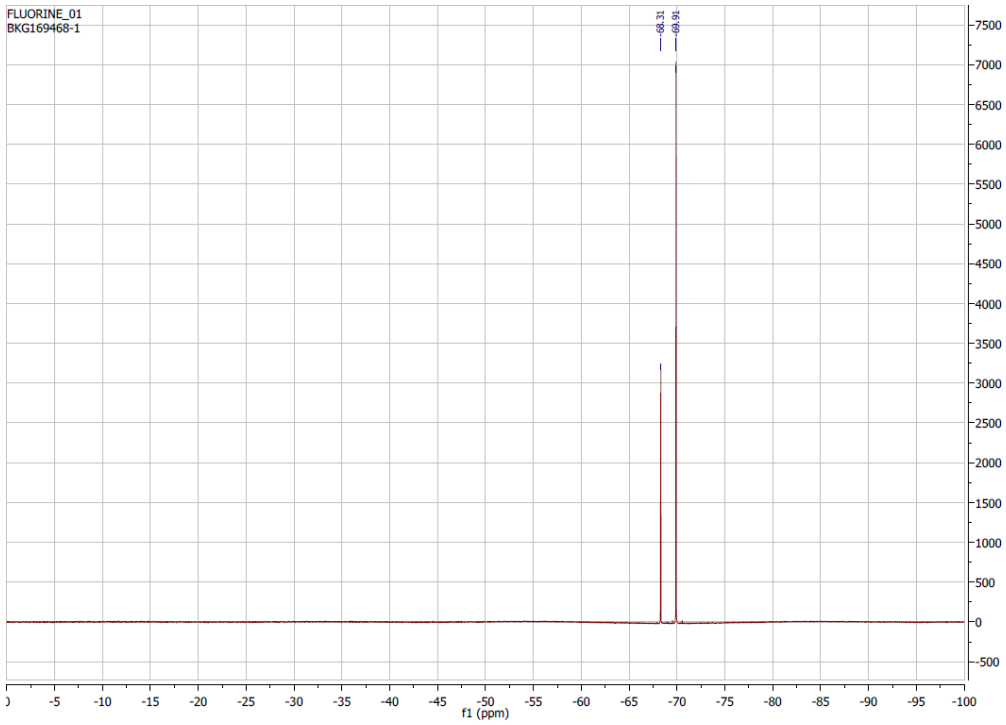


Figure S12. 1D ^{19}F NMR spectrum of pure **6** ($CDCl_3$, 282 MHz).

***Z*-endoxifen**

(*E*)-4-(2-phenyl-1-(4-(2-(2,2,2-trifluoro-*N*-methylacetamido)ethoxy)phenyl)but-1-en-1-yl)phenyl pivalate (56.8 g, 103 mmol) was dissolved in 1 L of THF and 150 mL of methanol. The mixture was cooled to 0 °C and LiOH.H_2O (21.6 g, 0.51 mol) was added portion wise. The mixture was stirred for 3 hours at rt. NH_4Cl-sat (500 mL) was added and the mixture was extracted with EtOAc (2x). The combined organic layers were washed with $NaHCO_3$-sat and brine, dried over Na_2SO_4 and concentrated affording a crude material (42 g). From a previous batch, 6.8 g of crude *Z*-endoxifen was added and the combined solids were triturated twice with 200 mL Et_2O affording pure *Z*-endoxifen as a white solid (37 g, 83% - recalculated from total amount of starting materials). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.91 (t, *J* = 7.2 Hz, 3H), 2.48 (q, *J* = 7.5 Hz, 2H), 2.50 (s, 3H), 2.93 (t, *J* = 5.1 Hz, 2H), 3.96 (t, *J* = 5.4 Hz, 2H), 6.49 (d, *J* = 8.7 Hz, 2H), 6.71-6.75 (m, 4H), 7.02 (d, *J* = 8.7 Hz, 2H), 7.08-7.18 (m, 5H).

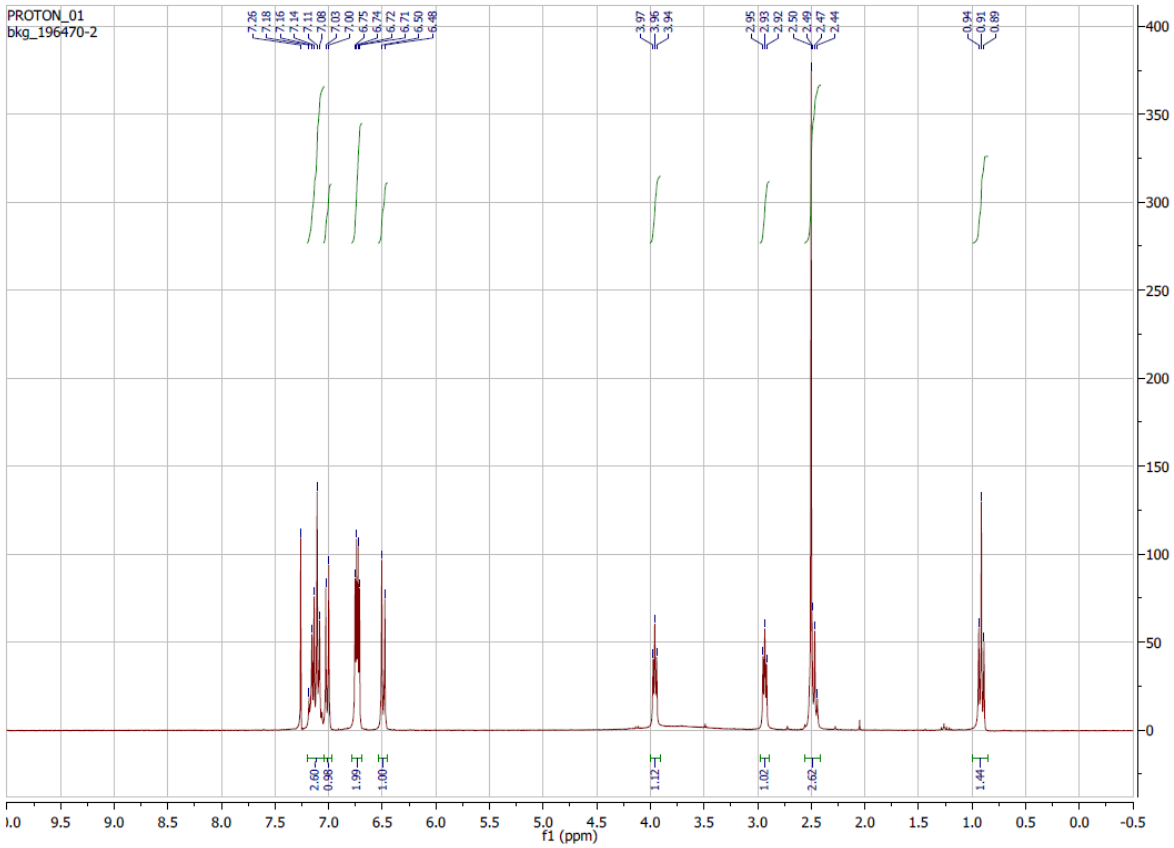


Figure S13. 1D ^{1}H NMR spectrum of pure *Z*-endoxifen ($CDCl_3$, 300 MHz).

qNMR-experiments

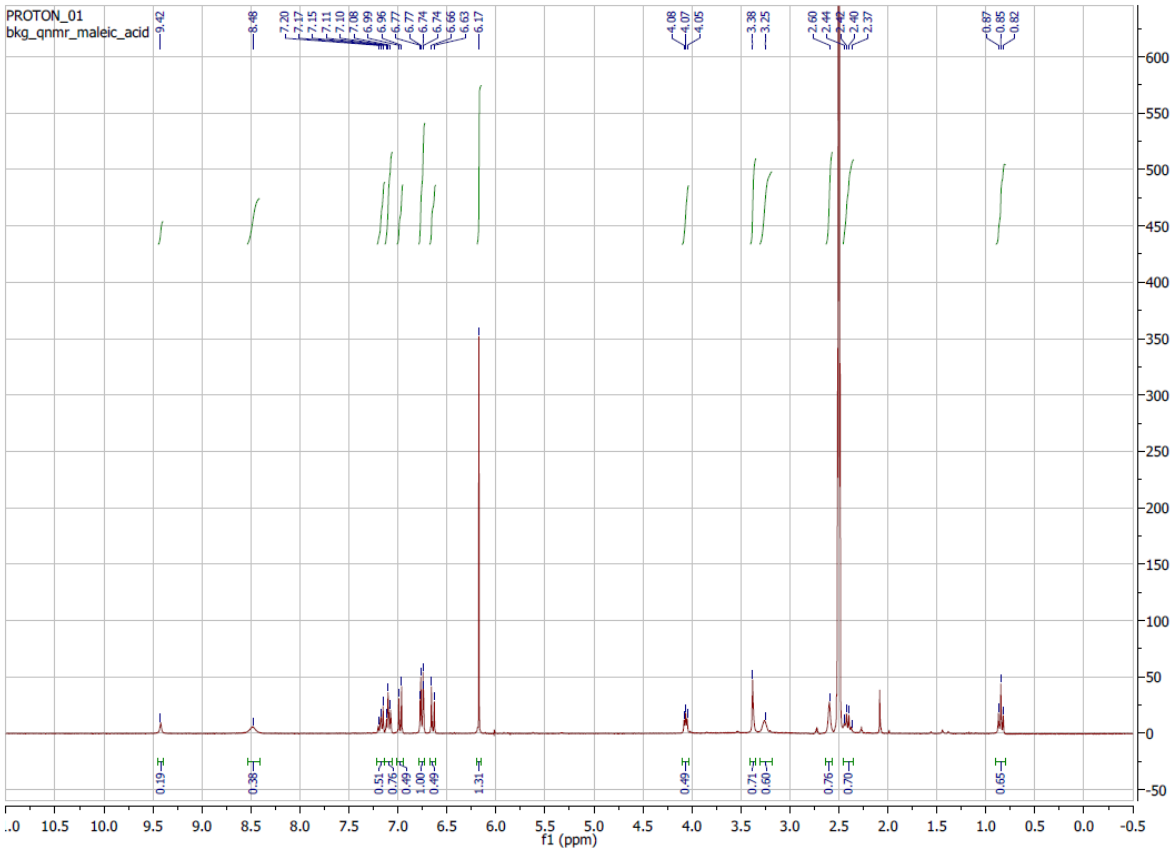


Figure S14. qNMR spectrum of maleic acid (11.31 mg) and *Z*-endoxifen (13.66 mg) in 2 mL of DMSO-*d6* (300 MHz) – purity 98.3%.

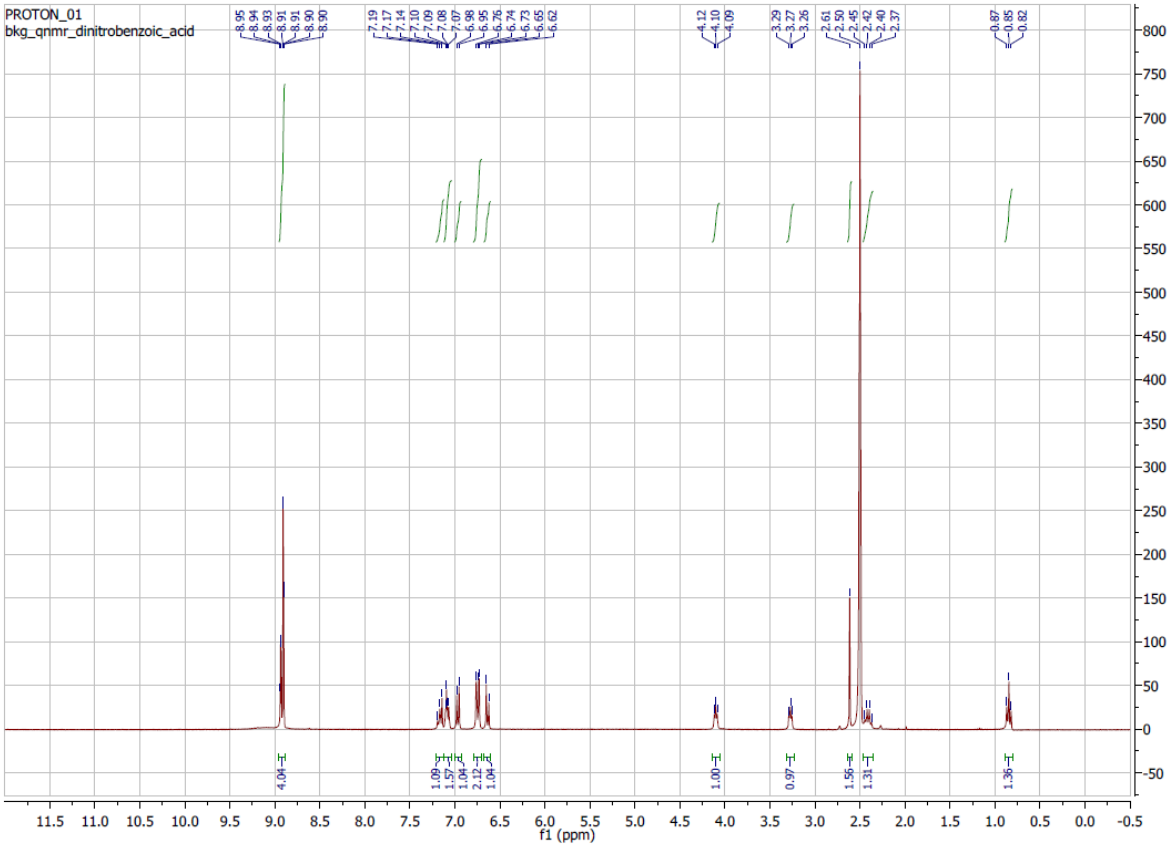


Figure S15. qNMR spectrum of 3,5-dinitrobenzoic acid (20.49 mg) and *Z*-endoxifen (14.35 mg) in 2 mL of DMSO-*d6* (300 MHz) – purity 97.8%.

Figure S16. LC-MS-UV analysis of *Z*-endoxifen.

Sample and Acquisition parameters

Sample and Acquisition Information

Sample Name: BKG196470-2
Date Acquired: 05/02/14
Used Method: Gradient B.lcm

Data file: BKG196470.lcd
Acquisition Time:12:41:10
Injection Volume: 2 ul

Comment:

System Parameters

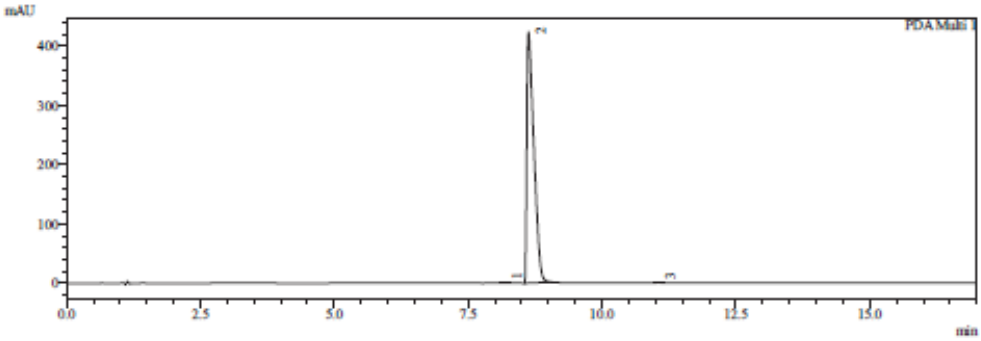
Probe type: ESI Type M

Flow: 1.000 ml/min
Buffer: B/C: 20.0/0.0 %
Concentration D: 20.0 %

Column Name: Luna 5u C18(2) 100A
Length x diameter - 100 x 4.6 mm

Oven Temperature: 40 °C

Chromatogram(s)



PDA Ch1 243nm 4nm

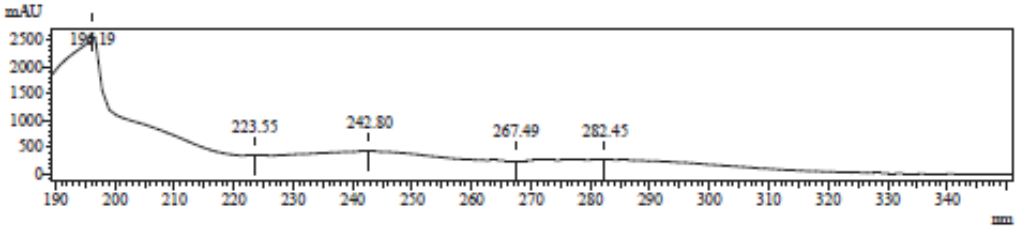
Peak#	Ret. Time (min.)	Area %
1	8.19	0.07
2	8.62	99.85
3	11.05	0.08
Total		100.00

PDA Ch2

PDA Ch3

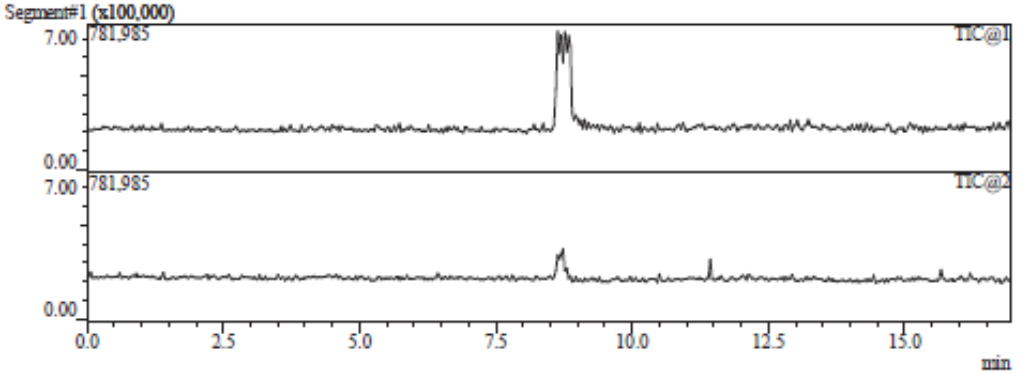
UV spectra

#: 1
Retention Time: 8.632
Maximum wavelength:196/243/282 nm



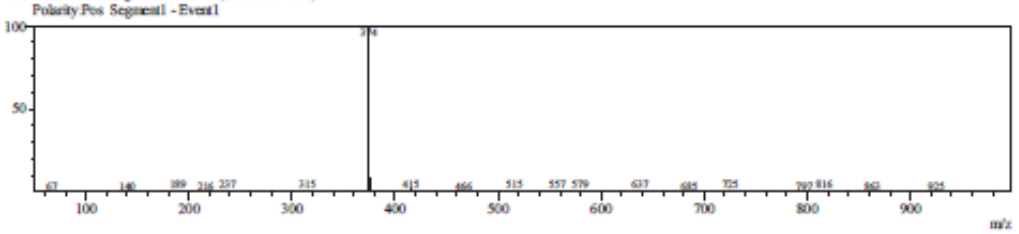
MS

TiC

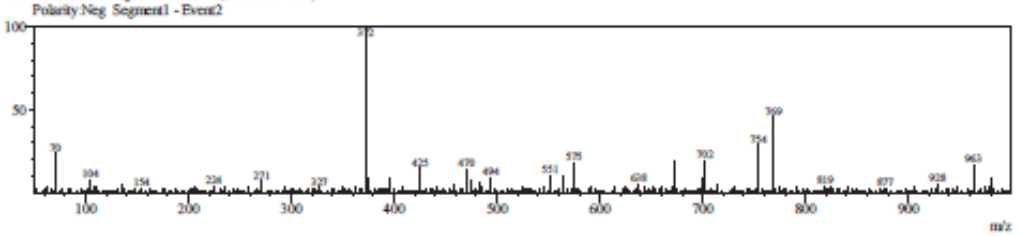


MS Spectrum Graph

#:1 Ret.Time:Averaged 8.800-8.867(Scan#:529-533)
Polarity:Pos Segment1 - Event1



#:2 Ret.Time:Averaged 8.817-8.883(Scan#:530-534)
Polarity:Neg Segment1 - Event2



Extended Method and Gradient Information

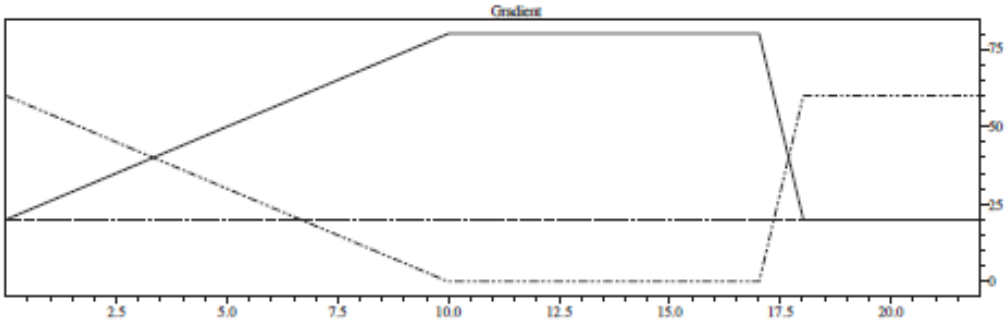
Mobile Phase Setup:
Mobile Phase A: Water
Mobile Phase B: Aqueous buffer pH 8
Mobile Phase C: Aqueous buffer pH 4
Mobile Phase D: Acetonitril

Pump Settings:

Pump Mode	:Low pressure gradient
Pump A	:LC-2010 Pump
Flow	:1.000 mL/min
B.Conc	:20.0 %
C.Conc	:0.0 %
D.Conc	:20.0 %
B.Curve	:0
C.Curve	:0
D.Curve	:0
PressMax	:250 bar
PressMin	:0 bar
LPGE Mode	:Auto

LC-Program:

Time	Unit	Command	Value	Comment
10.00	Pumps	Pump D Conc.	80	
17.00	Pumps	Pump D Conc.	80	
18.00	Pumps	Pump D Conc.	20	
22.00	Pumps	Pump B Conc.	20	
22.00	Pumps	Pump D Conc.	20	
22.00	Controller	Stop		



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