Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Full characterization of the conjugation adducts of the anti-Her2 antibody trastuzumab to 22-mer 5´-modified oligonucleotides

Supplementary Information

A. M. Carvalho,[a,b] A. Manicardi,[a] C. V. Montes,[b] S. B. Gunnoo,[a] R. J. Schneider,[b] and A. Madder*[a]

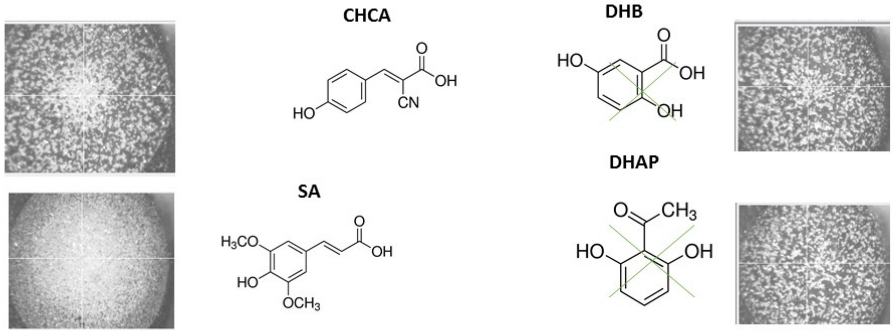

Figure S1. Co-crystallization patterns observed within different MALDI-ToF MS matrices.

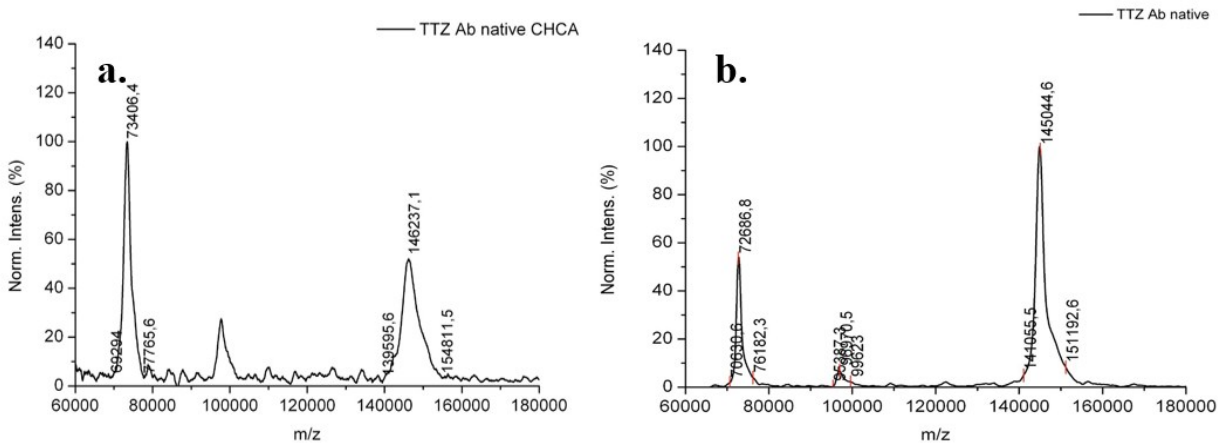

Figure S2. Smoothed MALDI-ToF MS spectra for Native TTZ Ab using a) CHCA matrix or b) SA matrix.

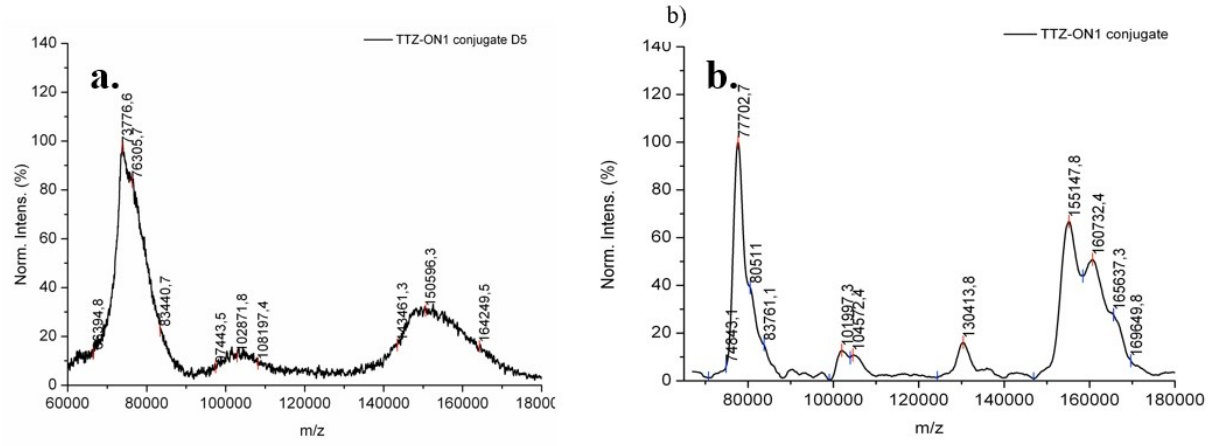

Figure S3. Smoothed MALDI-ToF MS spectra obtained for TTZ-ON1 conjugates using a) CHCA matrix or b) SA matrix.

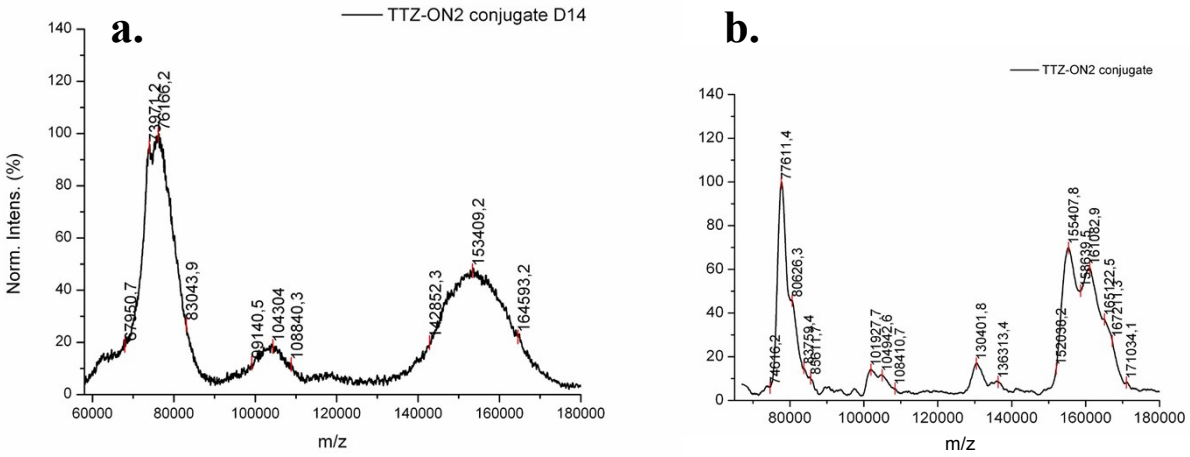

Figure S4. Smoothed MALDI-ToF MS spectra obtained for TTZ-ON2 conjugates using a) CHCA matrix or b) SA matrix.

Table S1. Determination of TTZ : ON ratio based on m/z differences between native TTZ and conjugates.

Native TTZ		TTZ-ON1 conjugate			TTZ-ON2 conjugate			
		m/z	TTZ-ON mass difference	TTZ:ON ratio		m/z	TTZ-ON mass difference	TTZ:ON ratio
$[M+2H]^{2+}$	72687	77703	5016	1.46	$[M+2H]^{2+}$	77612	4925	1.42
		80511	7824	2.23		80626	7939	2.30
		83761	11074	3.42		83759	11073	3.20
$[M+H]^{+}$	145045	155148	10103	1.46	$[M+H]^{+}$	155408	10363	1.50
		160732	15688	2.27		161083	16038	2.32
		165637	20593	2.98		167211	22166	3.21

Table S2. PBS 100 mM buffer composition.

Salt	mass for 1L solution	Final concentration (mM)
NaCl	74 g	1.27 mM
Na_2HPO_4	9.94 g	70 mM
$NaH_2PO_4.H_2O$	4.15 g	30 mM
EDTA	5.845 g	20 M

Preparation of SDS denaturing gradient gels (4-10%)

Table S5 summarizes the components used in the preparation of a 4-10% gradient gel. First the 10% resolving gel was prepared by mixing the ingredients in Table S2. TEMED was added last and immediately poured into the glass frames up to the middle. A solution of milliQ water:isopropanol 50:50 was added in order to later allow the second resolving gel (4%) to merge with the first gel (10%). While the 10% gel was polymerizing, the 4% gel was prepared, adding TEMED at last and pouring the mixture on the glass frames up to the top trying to avoid air bubbles. Combs were inserted (1 mm, 10 wells) and the gel was allowed to polymerize for approximately 1h.

Table S3. Ingredients used in the preparation of a denaturing 4-10% discontinuous gel.

Ingredients	10 % (resolving)	4 % (stacking)
milliQ-H_2O	8.7 mL	12.0 mL
10 % (w/v) SDS	200 µL	200 µL
Tris-acetate 3 M pH 7.0	5.0 mL	5.0 mL
Acrylamide 29:1	6 mL	2.66 mL
APS 10 %	100 µL	100 µL
TEMED	15 µL	20 µL
Final Volume	20 mL	20 mL

Table S4. Ionization parameters for MALDI-ToF MS characterization of TTZ-ON conjugates.

Detector gain	51x1999 V
Smart beam parameter Set Frequency	66.7 Hz
Sample Rate and Digitizer Settings	0.05 GS/s
Ion Suppression up to	< 60000 Da
Pulsed Ion Extraction	2000 ns