Electronic supporting information

Investigation on the reactivity of nucleophilic radiohalogens with arylboronic acids in water: access to an efficient single-step method for the radioiodination and the astatination of antibodies

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1. Organic synthesis

Preparation of (3-(*N*-hydroxysuccinimidyl)carbonyl)phenyl)boronic acid (4).

To a solution of 3-boronobenzoic acid (3 mmol, 1 eq) in dry DMF (25 mL) was added EDCI (4.5 mmol, 1.5 eq), *N*-hydroxysuccinimide (4.5 mmol, 1.5 eq) and triethylamine (27 mmol, 9.0 eq). The solution was stirred for 28 h at room temperature. The solvent was evaporated *in vacuo* and the obtained residue was dissolved in CH_2Cl_2. 1N HCl was then added to the solution and the aqueous layer was extracted three times with CH_2Cl_2. The organics layers were dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The crude product was then purified on a silica gel column using MeOH (0 % to 1.5 %) in CH_2Cl_2 to provide **4** as a white solid (123 mg, 45% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.53 (s, 1H), 8.42 (s, 2H), 8.20 (d, J = 7.4 Hz, 1H), 8.12 (d, J = 8.0 Hz, 1H), 7.63 (t, J = 7.7 Hz, 1H), 2.90 (s, 4H); ^{13}C NMR (100 MHz, DMSO) δ 170.33, 162.07, 140.92, 135.55, 131.41, 128.60, 123.79, 25.53.

2. NMR spectra

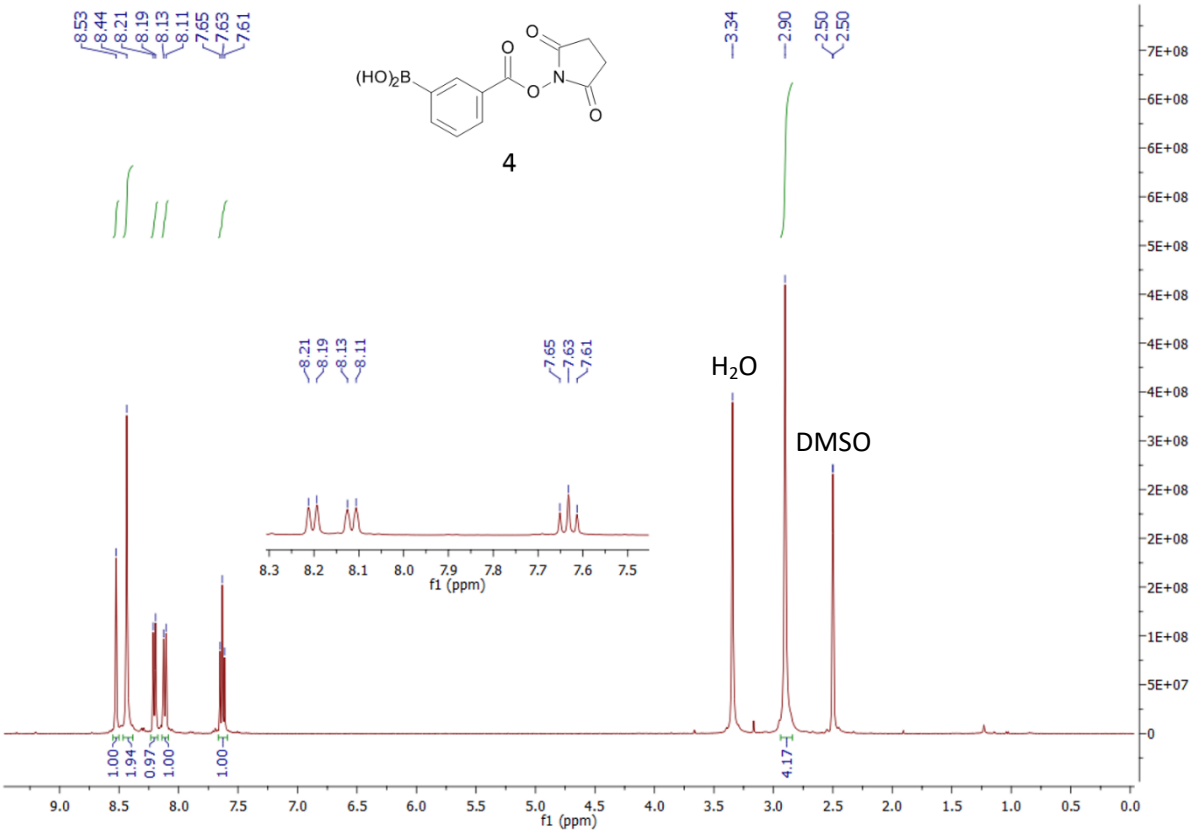

Fig. S1. ^{1}H NMR spectra of 3-(N-hydroxysuccinimidyl)carbonyl)phenyl)boronic acid (**4**) in DMSO-d_6

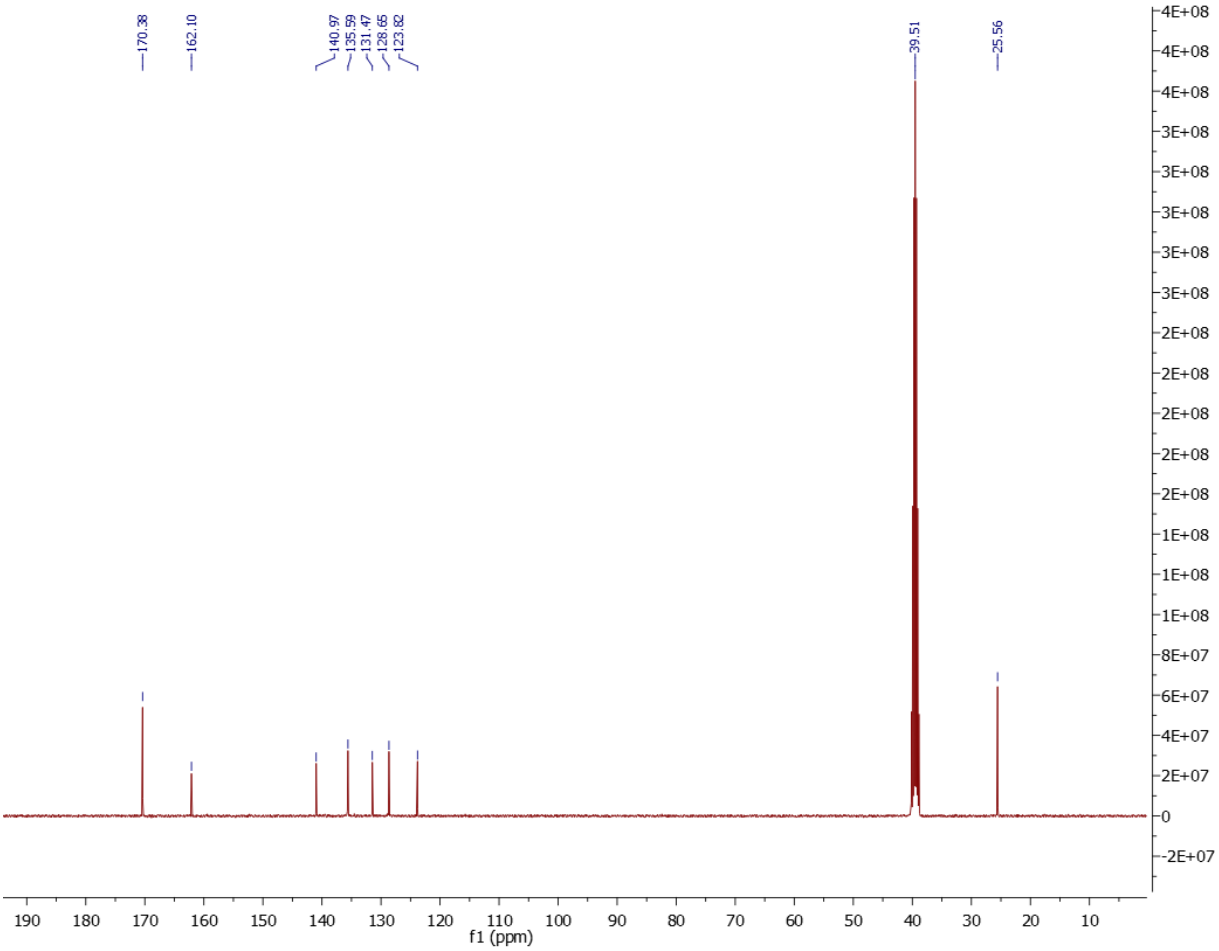

Fig. S2. ^{13}C NMR spectra of 3-(N-hydroxysuccinimidyl)carbonyl)phenyl)boronic acid (**4**) in DMSO-d_6

3. HPLC UV- and radio-chromatograms

Radiolabelling products of (**1**) were identified by HPLC analyses of the cold reference 1-chloro-4-iodobenzene. Since astatine and iodine have similar polarity, the difference of retention time between the astatinated and iodinated product was small. Radioiodination and astatination reactions were analysed on two different HPLC systems using the same configuration which explain the difference in retention time between Fig. S3 and S4.

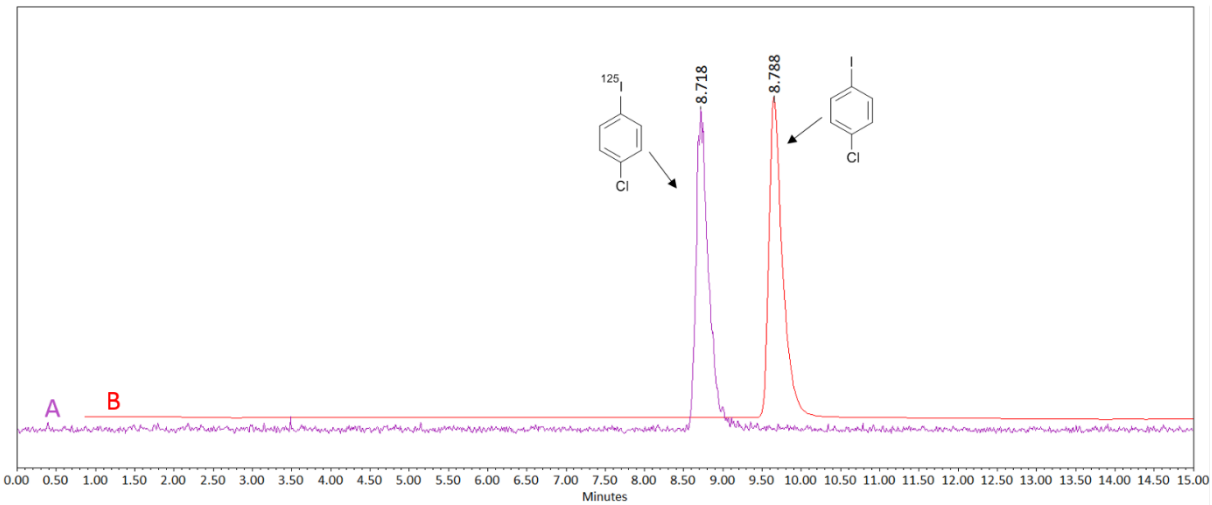

Fig. S3. Radiolabelling of (1) with UV reference of the cold product A) ^{125}I-iodination of (1); B) UV analyse (254 nm) of the cold reference for radioiodination.

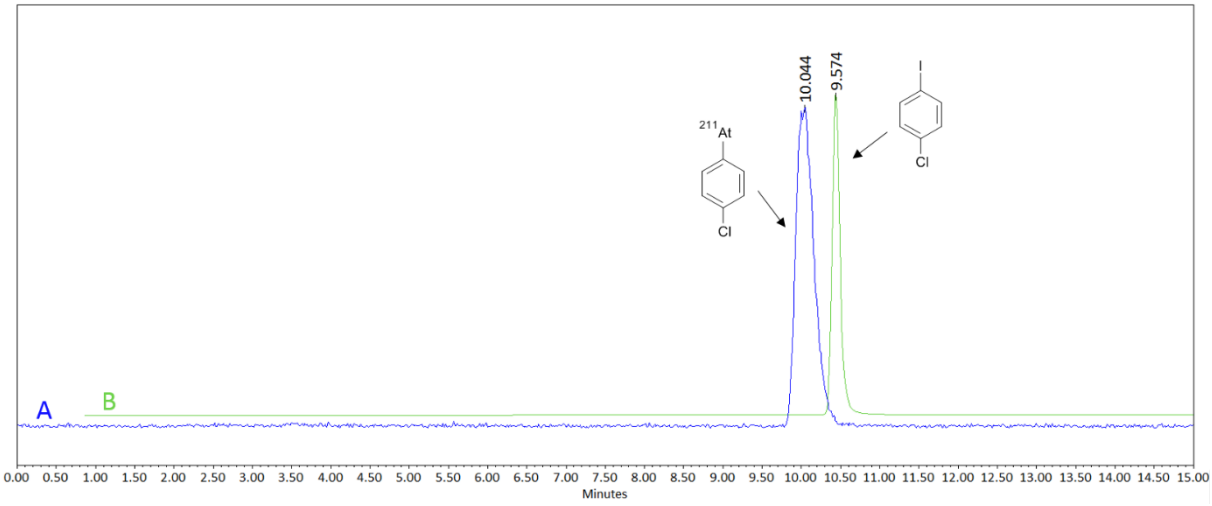

Fig. S4. Radiolabelling of (1) with UV reference of the cold product A) ^{211}At-astatination of (1); B) UV analyse (254 nm) of the cold reference for astatination.

Calculation of RCYs

The calculation of RCYs were calculated as followed: an aliquot of the crude product was injected on HPLC (Fig. S4 A). At the end of this run, an injection of a 10 mg/mL sulfite solution (50 µL) is performed (Fig. S3 B) in order to remove all astatine species remaining on the column. The activity detected during this run was taken into account as unlabelled ^{211}At in the calculation of RCYs by subtraction of the area of the peak. Correction decay between the two runs was not considered for the calculation of the RCYs as only 10 minutes separated the two pics, which was negligible regarding the half-life of ^{211}At. For example, the calculated RCY presented in Fig. S5 is 8 % and not 14.4 % if considering radiochromatogram A only.

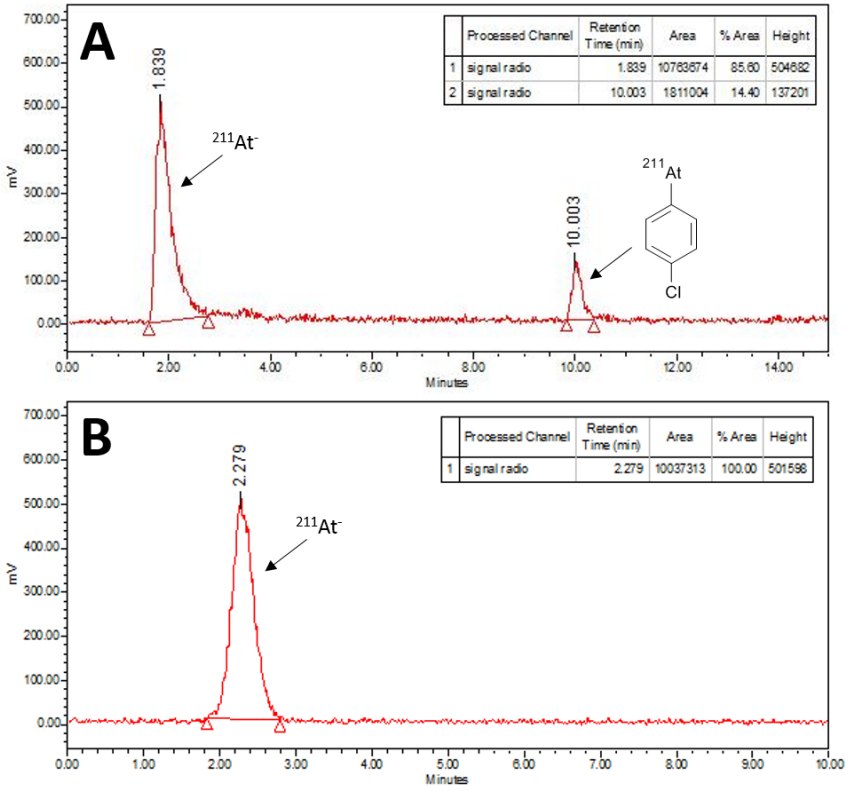

Fig. S5. RadioHPLC analyses of the astatination of (**1**) at pH 8. A) radiochromatogram of the radiolabelling; B) radiochromatogram of the sulfite run performed after the HPLC-analyse of the radiolabelling.

4. Radio-TLC analyses

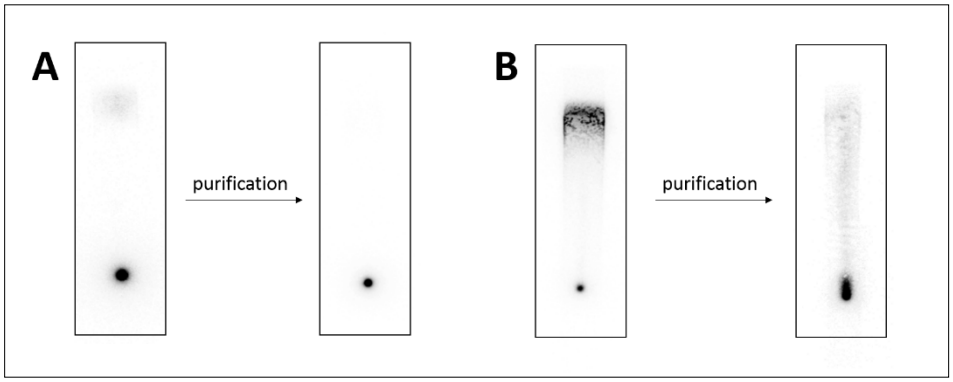

Fig. S6. ITLC-SG analyses (MeOH as eluent) of A) ^{211}At-astatinated aBA-9E7.4 before and after purification on PD-10 column; B) ^{211}At-astatinated unmodified 9E7.4 (i.e. non-specific binding) before and after purification on PD-10 column.

5. Mass spectrometry analyses

Antibodies were conditioned in PBS with a concentration set at 200 µg/mL. Desalting and separation of proteins were achieved on an H-Class UPLC system (Waters Corporation, Milford) by injection of 10 µL of solutions onto an Acquity® BEH300 C4 column (2.1 mm × 50 mm, 1.7 µm; Waters Corporation) held at 60 °C. The mobile phase was composed of 5 % acetonitrile as solvent A and 100 % acetonitrile as solvent B, each containing 0.1 % formic acid. The elution was carried out using a gradient of solvent B in solvent A over 12 min at a constant flow rate of 400 µL/min (from 0 to 8 min: from 5 % to 95 % of solvent B, from 8 to 10 min: kept constant at 95% of solvent B, from 10 to 12 min: from 95 % to 5 % of solvent B). High-resolution mass spectrometry (HRMS) detection of proteins was performed by a Synapt G2 HRMS Q-TOF mass spectrometer equipped with a Z-Spray interface for electrospray ionization (Waters Corporation). The resolution mode was applied in a mass-to-charge (m/z) ratio ranging from 200 to 4,000 at a mass resolution of 25,000 Full Width Half Maximum in the positive ionization mode. Ionization parameters were as follow: capillary voltage of 3 kV, cone voltage of 30 V, desolvatation gas flow of 900 L/hr, source temperature of 120 °C, desolvatation temperature of 450°C, Nitrogen as desolvatation gas. Data were collected in the continuum mode at a rate of four spectra per second. Leucine enkephalin solution prepared at 2 µg/mL in an acetonitrile/water (50/50, v/v) mixture was infused at a constant flow in the lock spray channel. A spectrum of 1 s was acquired every 20 s and allowed mass correction during experiments. The experimental molecular weights of proteins were finally deducted by deconvolution with the MaxEnt1 extension of MassLynx® software (version 4.1, Waters Corporation). Typical analyses used for the determination of the aBa/mAb ratio are given on Fig S7.

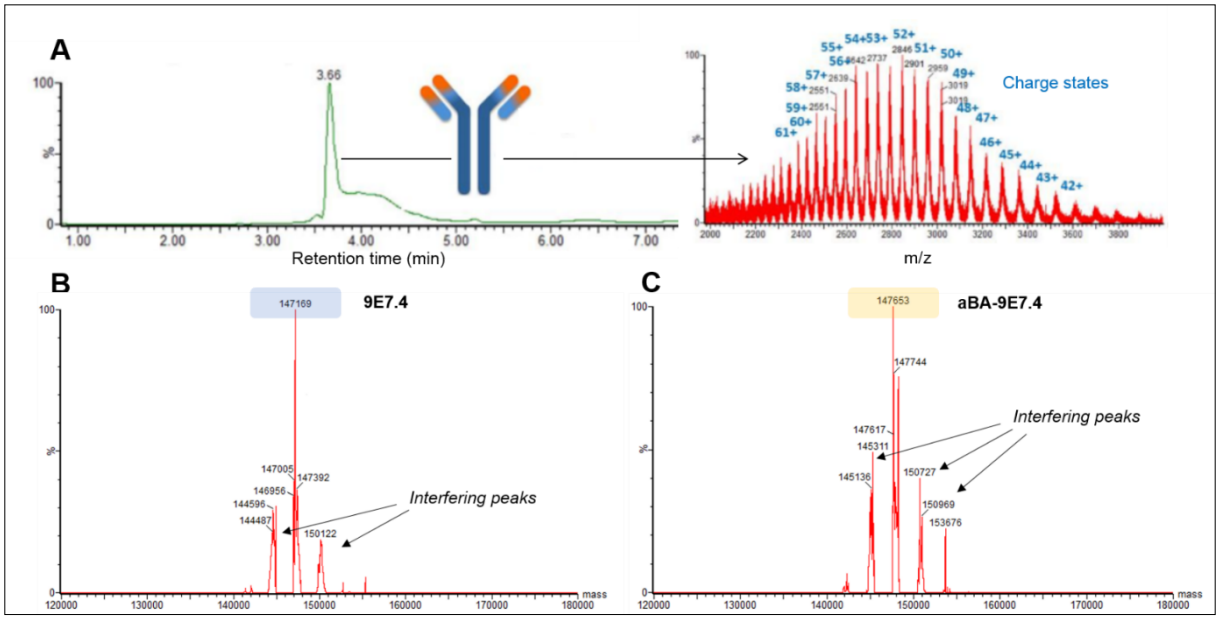

Fig. S7. Mass spectrometry analysis of 9E7.4 and aBA-9E7.4 mAbs. A) Representative chromatogram and related mass spectrum of 9E7.4. B-C) Calculated molecular weights of 9E7.4 and aBA-9E7.4 from the deconvolution (MaxEnt1 software, Waters Corporation) of related mass spectra. In this example, the mass difference is 484 g/mol, corresponding to 3.3 aBA/mAb.