

Multivalent Poly(ethylene glycol)-Containing Conjugates for *In Vivo* Antibody Suppression (Supporting Information)

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Characterization of conjugates and estimates of purity.

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The purities of the conjugates were determined using analytical HPLC using a weak cation exchange column. The domain 1 polypeptide has an isoelectric point greater than 9, and cation exchange was the only method tried which satisfactorily resolved the multivalent PEG conjugates from other products of the conjugation reaction. By-products included conjugates with lower substitution such as conjugates with only three, two, or one polypeptides attached. Leftover TA/D1 was also included in the mixture, as were other minor impurities. The purity of conjugates is not expected to significantly influence the ability to suppress antibody. Typical purity of TA/D1 used in preparing the conjugates was 83-90% as determined by reverse phase HPLC. Domain 1 polypeptide of 16 additional mass units, due to oxidation of methionine to methioninesulfoxide, accounted for 5-10% of the material. The remaining 5-7% of the material was unidentified. Some of the impurities are undoubtedly carried through the preparation of the conjugates. The purities and mass spectral data of the conjugates are presented in Table 1. Purities are defined as the percentage of total area associated with tetravalent conjugates eluting from an analytical weak cation exchange HPLC column.

Table 1. Purities and Mass Spectral Data of Conjugates

Compound	HPLC purity			MALDI mass spec	
	method ^a , gradient ^b	elution time	purity ^c	calculated	found
<u>1a</u> (LJP 1027)	WCX, 0-25 %B	18.2 min	98 %	29,783	29,783
	RP, 25-45 %B	16.8 min	99 %		
<u>1b</u> (LJP 1078)	WCX, 0-20 %B	18.3 min	99 %	34,666	35,025
<u>1c</u> (LJP 1081)	WCX, 0-20 %B	16.1 min	99 %	41,143	41,922
<u>1d</u> (LJP 1077)	WCX, 0-20 %B	15.1min	96 %	51,802	51,636
<u>1e</u> (LJP 1086)	WCX, 0-20 %B	13.9 min	99 %	61,803	61,739
<u>1f</u> (LJP 1084)	WCX, 5-25 %B	17.7 min	98 %	81,638	81,893
<u>1g</u> (LJP 1082)	WCX, 0-20 %B	13.4 min	98 %	50,600	51,620
<u>1h</u> (LJP 1083)	WCX, 0-16 %B	13.1 min	99 %	48,898	49,390

^a WCX (weak cation exchange) chromatography was performed on a 4.0 x 250 mm Propac WCX column (Dionex) with a flow rate of 1 mL/min, A buffer = 90/10 10 mM NaH₂PO₄ pH 7/acetonitrile, B buffer = A + 1 M NaCl; RP (reverse phase) chromatography was performed on a 4.6 x 25 mm diphenyl column (Vydac) with a flow rate of 1 mL/min, A buffer = 0.1 % TFA/water, B buffer = 0.1 % TFA/acetonitrile. ^b Gradients are linear over 20 minutes with beginning and ending percentage of B buffer listed. ^c Percentage of total area associated with tetravalent conjugate.