Preparation of Biphenyl-Conjugated Bromotyrosine for Inhibition of PD-1/PD-L1 Immune Checkpoint Interactions

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Analytical data
^{1}H NMR spectra

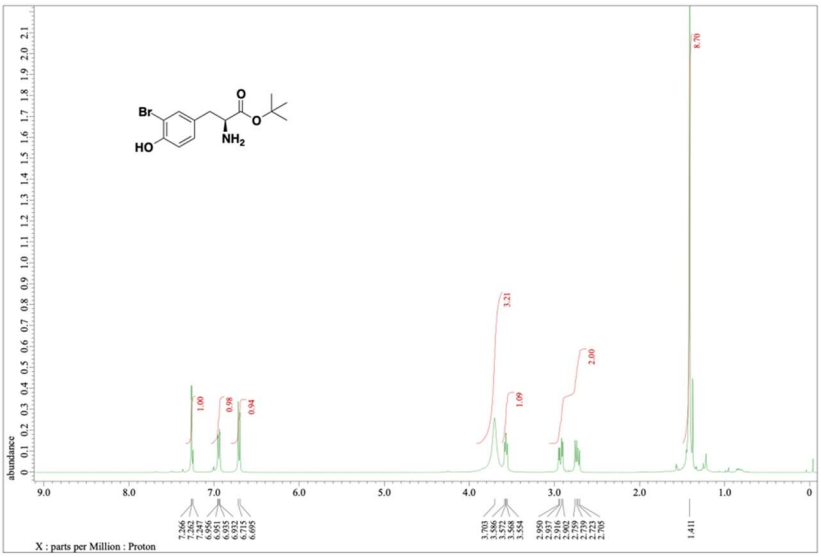

Figure S1. ^{1}H NMR spectrum of **3** in $CDCl_3$.

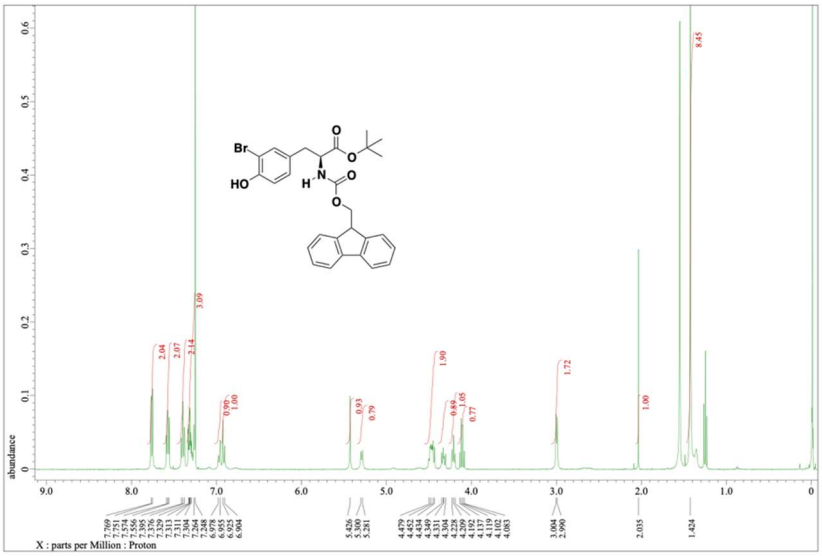

Figure S2. ^{1}H NMR spectrum of **4** in $CDCl_3$.

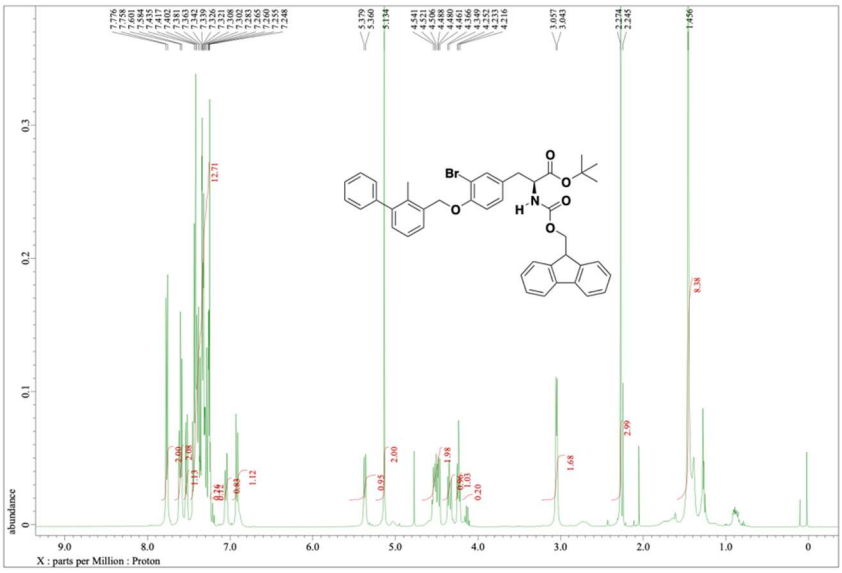

Figure S3. ^{1}H NMR spectrum of **5** in $CDCl_3$.

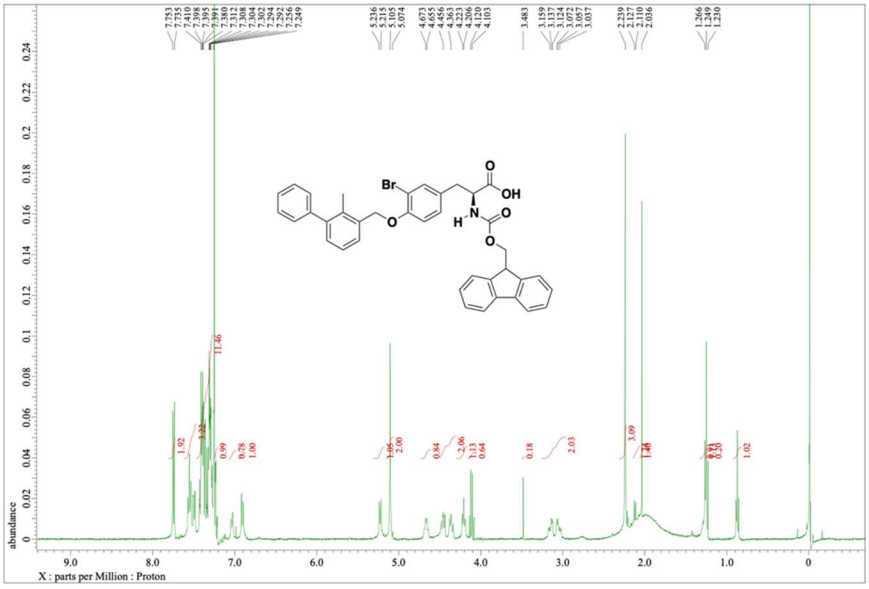

Figure S4. ^{1}H NMR spectrum of **6** in $CDCl_3$.

Table S1. Summary of molecular weights and HPLC elution times for the synthesized compounds.

Sequence	[a][M +H]$^+$	[b]t (min)	Sequence	[a][M +H]$^+$	[b]t (min)
X	440.1	15.8	**GXG**	554.1	13.0
GX	497.0	14.6	**XNL**	667.2	16.4
XG	497.1	18.8	**XNH**	691.2	14.9
XS	527.1	16.0	**XHP**	674.2	14.1
XR	586.1	18.2	**XGG**	554.1	16.4
XA	511.1	19.0	**XCSE**	759.4	15.1
XW	626.2	15.5	**XGGG**	611.1	19.3
YXC	706.2	17.3	**WRXNN**	1010.3	18.1
WXG	683.2	19.1	**ERXNK**	967.3	12.0
QXQ	696.2	14.4	**WRXNQ**	1024.4	13.4
NXR	710.2	18.2	**XRRRR**	1064.5	15.7
CXA	614.1	14.9	**XGGGG**	668.2	19.3
RXN	710.2	11.7	**XGGGGG**	725.3	18.4
SXR	683.2	18.4	**CERXNKM**	1201.4	15.6
CXR	699.2	14.8	**FWRXNNI**	1270.5	19.3

[a]Determined by MALDI-TOF MS. Matrix: 2,5-dihydroxybenzoic acid.

[b]Estimated by HPLC using an InertSustain C18 column and 35–65% acetonitrile containing 1% trifluoroacetic acid as the eluant. Letters represent the single letter amino acid code.

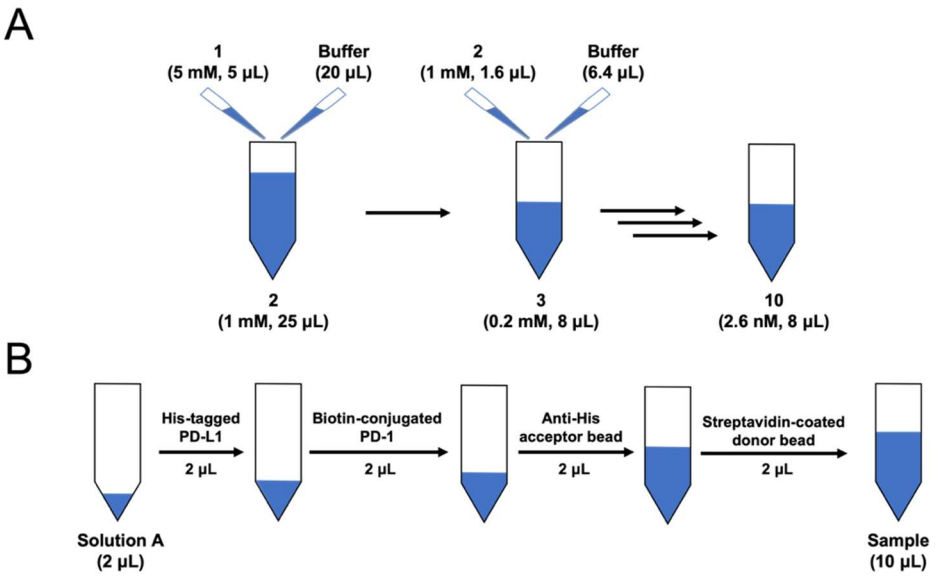

Figure S5. Preparation of (**A**) sample dilutions and (**B**) reaction samples.

Table S2. Concentrations of inhibitors in stock solutions (A) and in the assay sample.

	1	2	3	4	5	6	7	8	9	10
Solution A	5.0 mM	1.0 mM	0.2 mM	40 µM	8.0 µM	1.6 µM	0.32 µM	64 nM	13 nM	2.6 nM
Sample	1.0 mM	0.2 mM	40 µM	8.0 µM	1.6 µM	0.32 µM	64 nM	13 nM	2.6 nM	0.5 nM

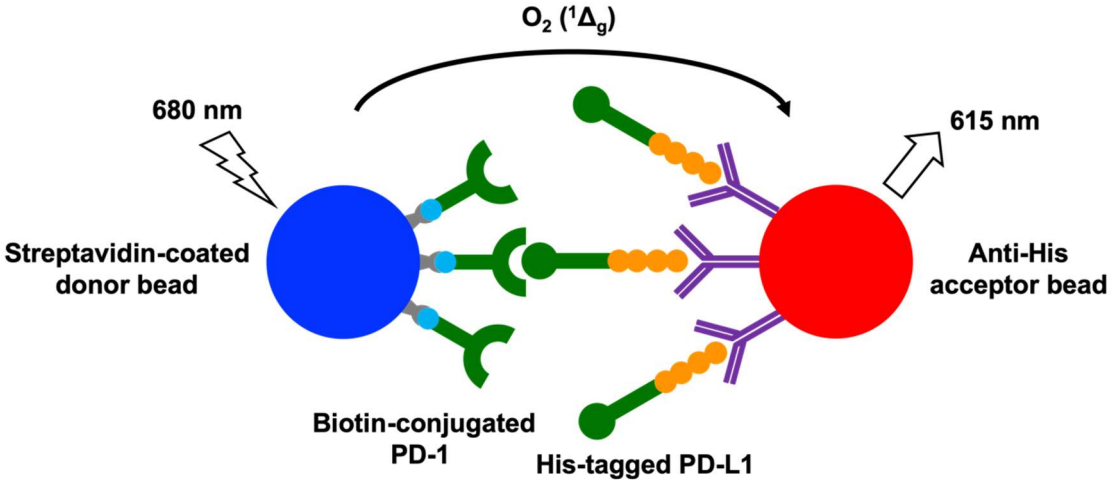

Figure S6. Principle of the Amplified Luminescence Proximity Homogeneous Assay (Alpha) assay for detection of PD-L1/PD-1 interactions through photoinduced energy transfer. Fluorescence intensity at 615 nm increases upon binding of PD-L1 to PD-1 and is reduced by competitive inhibition in the presence of **X** or **amino-X** compounds.