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Supplemental information

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Supporting Information

Dual-pharmacophore artezomibs hijack the *Plasmodium* ubiquitin-proteasome system to kill malaria parasites and overcome resistance

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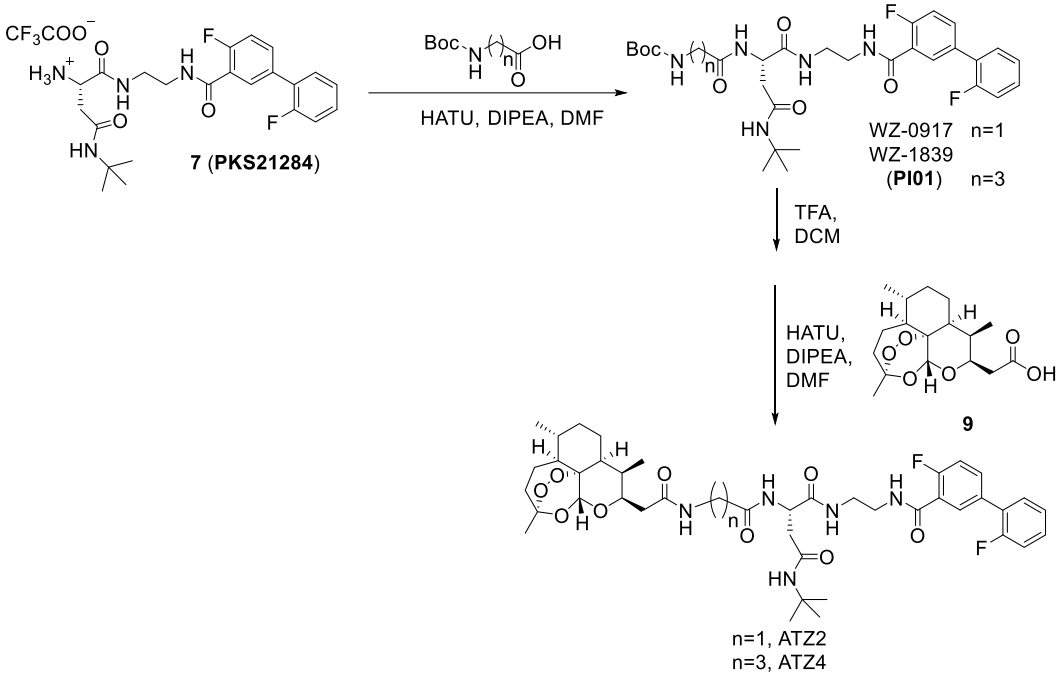
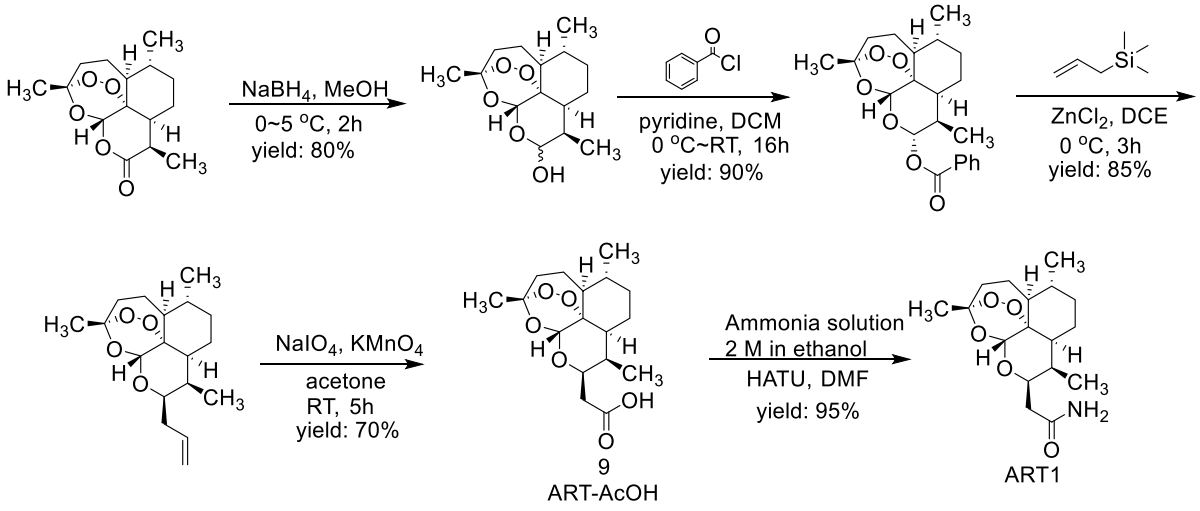
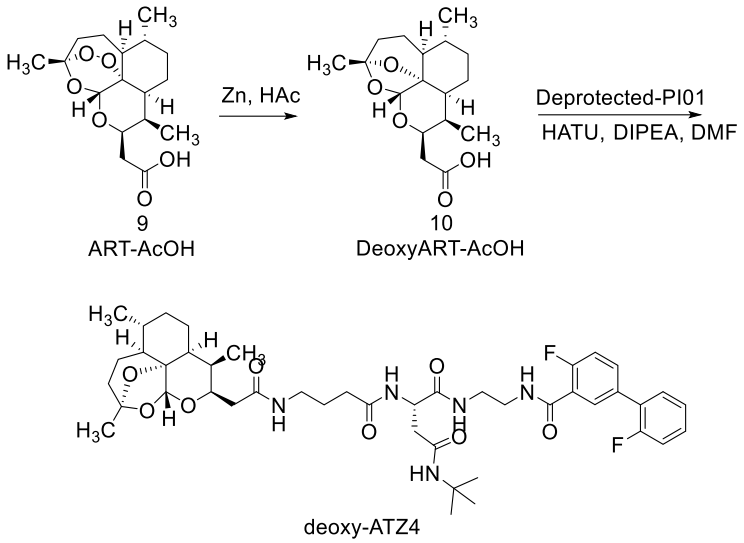


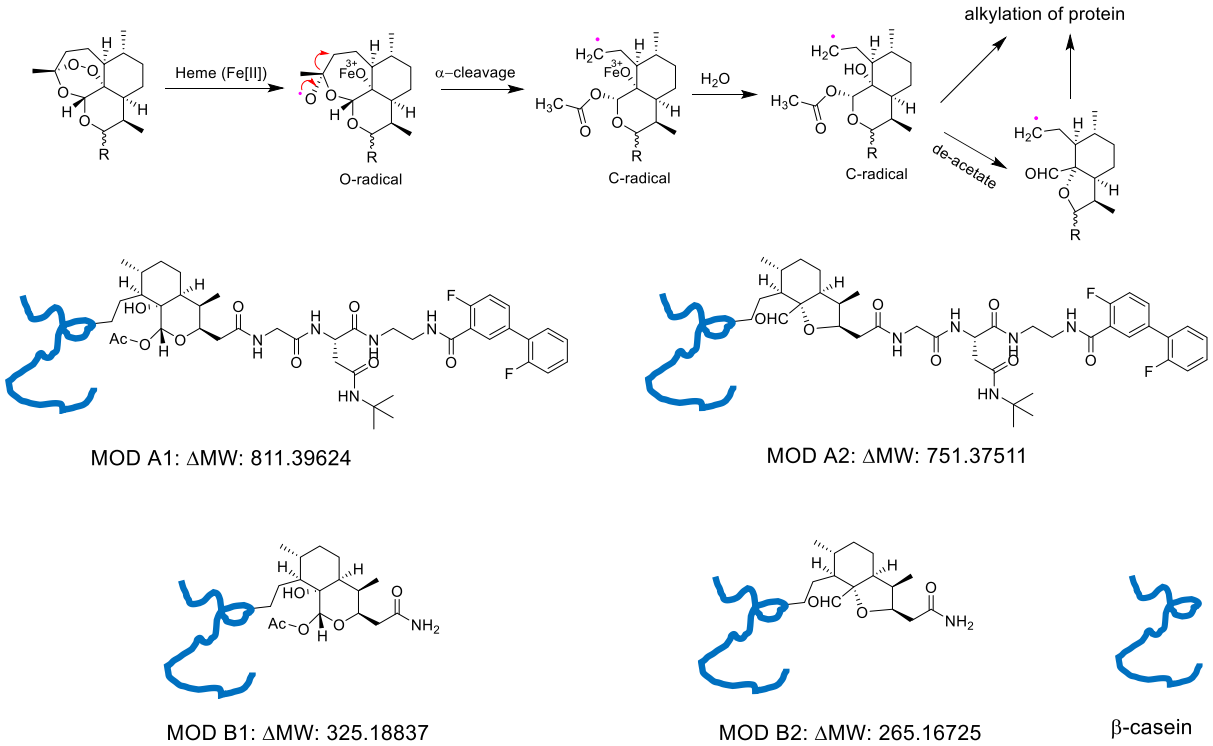
Scheme S1. Synthetic route of ART-based hybrids ATZ2 and ATZ4. Related to Figure 1A.



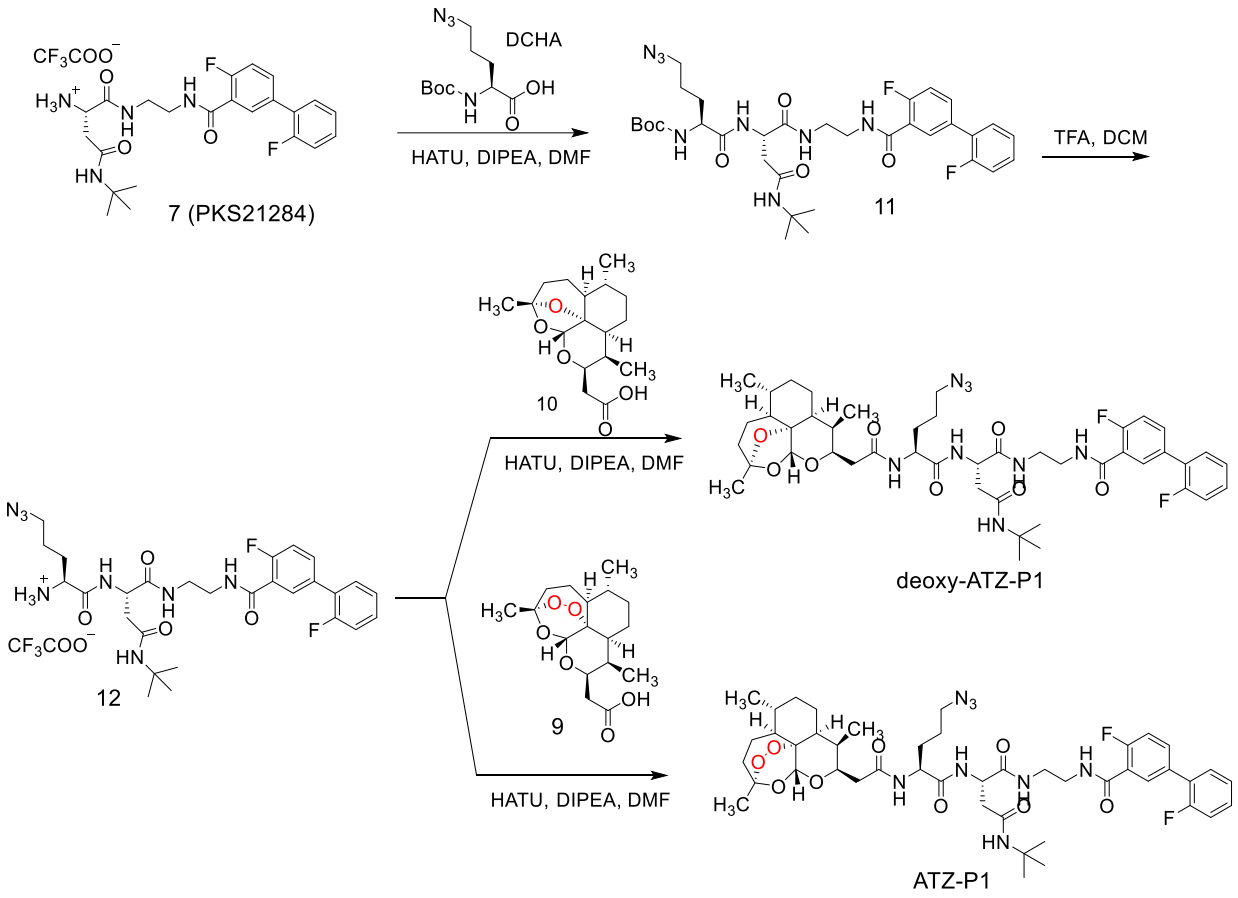
Scheme S2. Synthetic route of ART-AcOH (9) and ART1. Related to Figure 1A.



Scheme S3. Synthesis of deoxy-ATZ4. Related to Figure 1A.



Scheme S4. Heme-induced activation of the endoperoxides, yielding reactive radical intermediates of ART1 and ATZ2 capable of two types of covalent modification of β-casein. Related to Figure 2.



Scheme S5. Synthesis of ATZ-P1 and deoxy-ATZ-P1. Related to Figure 5A.

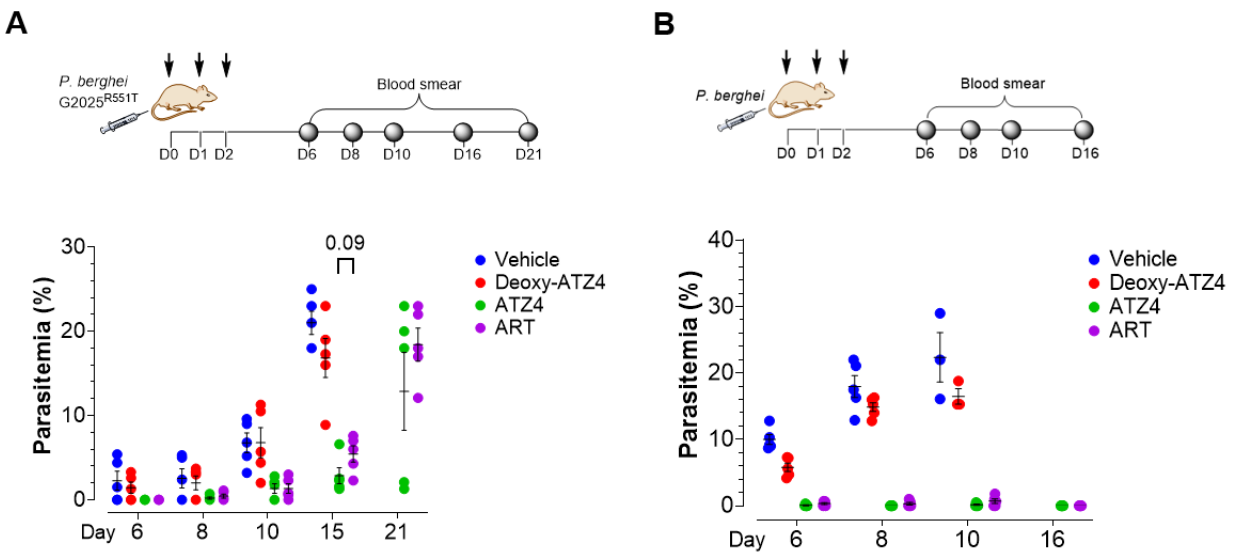


Figure S1. In vivo efficacy of ATZ4 in mouse models of recrudescence and Pb infection. **(A)** Second recrudescence experiment. Mice infected with Pb ART resistant K13^{R551T} mutant were treated with vehicle, ART (50 mg/kg), ATZ4 (120 mg/kg) and deoxy-ATZ4 (120 mg/kg for 1st experiment and 120 mg/kg for 2nd experiment) administered via i.p. 3 hours post inoculation of parasites on day 0 and continued on day 1 and day 2 (shown by arrows). Parasitemia in each mouse was monitored by microscopic analysis of Giemsa-stained blood smears on day 6, 8, 10., 16 and 21. The rates of recrudescence in each cohort on the indicated days were plotted as the percentages of mice with a positive smear. (**B**) In vivo efficacy of ATZ4 in mice infected with *Pb* wild type, in comparison with vehicle, deoxy-ATZ4, and ART. Related to Figure 1D.

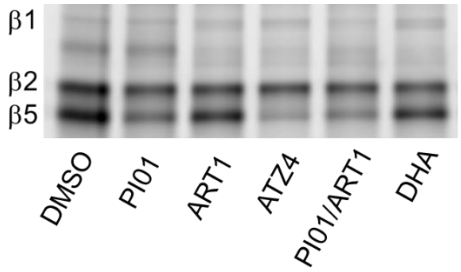


Figure S2. Labelling inhibition of *Pf*20S in Dd2 parasites treated with DMSO, PI01, ART1, ATZ4, PI01/ART1 (1:1) or DHA, assessed by their ability to block labeling of the parasites' proteasomes by MV151. Parasites were treated with indicated compounds for 6 hours and extracellular compounds were removed prior to hypotonic lysis of red blood cells. Related to Figure 5C-D.

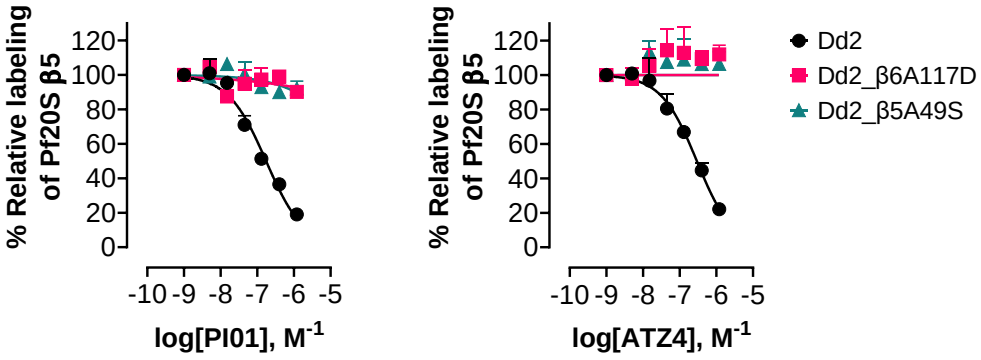


Figure S3. Inhibition of labeling of the Pf20S β5 by PI01 and ATZ4 in lysates of Dd2, Dd2β6^{A117D}, and Dd2β5^{A49S}. Densitometry analyses of the β5 bands in Figure 4 and extended raw data **d**. were done with ImageJ. Related to Figure 4 and extended raw data d.

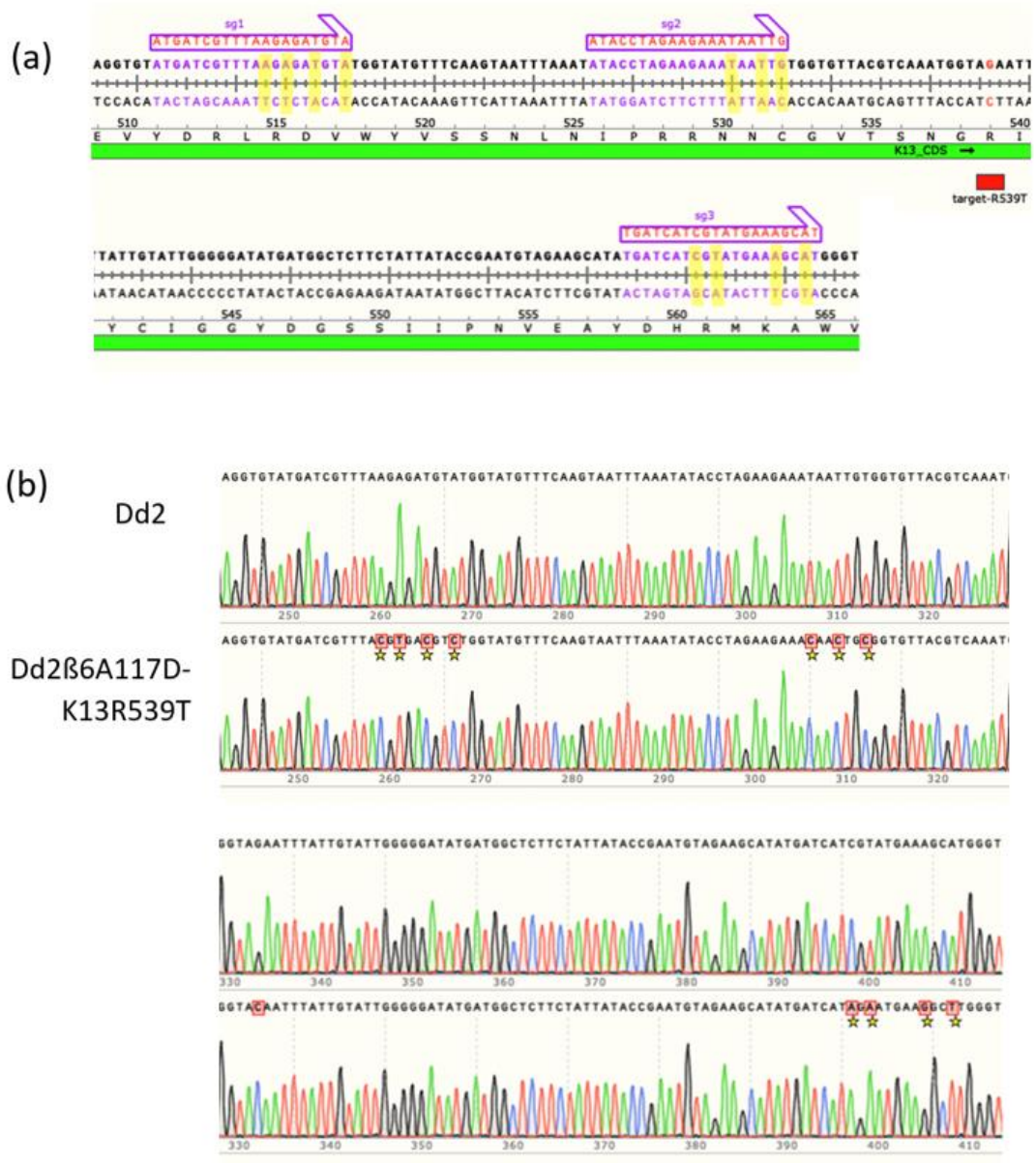


Figure S4. sgRNA:Cas9 mediated single nucleotide replacement in K13 gene to generate K13R539T mutation in Dd2ß6A117D cell line. (a) K13 locus showing the target 20-nucleotide guide sequence (purple) recognized by sgRNA:Cas9. In order to have a robust Cas9 editing, three different single guides were selected (sg1, 2 and 3). To prevent cleavage of the modified locus by Cas9, shield mutations (highlighted in yellow) were incorporated into the repair template. Target single nucleotide replacement to achieve R539T is highlighted in red. (b) Chromatogram of the modified locus in Dd2ß6A117D showing the desired mutation and shield mutations (yellow stars). Dd2 sequence serves as a non-edited gene sequence control. Related to Figure 6A.

Table S1. Two types of modification of β-casein by ATZ2 or ART1 as shown in **Scheme S6**. (A) For ATZ2, MOD A_1 Δmass = 811.39624 and MOD A_2 Δmass = 751.37511 (A_1-acetate); (B) For ART1, MOD B_1 Δmass =325.18837 and MOD B_2 Δmass =265.16725 (B_1-acetate). Related to Figure 2C-D.

Tagged molecules	Modification	Molecular formula	Theoretical mass (monoisotopic)
ATZ2	MOD A_1	$C_{42}H_{55}F_2N_5O_9$	811.39624
	MOD A_2	$C_{40}H_{51}F_2N_5O_7$	751.37511
ART1	MOD B_1	$C_{17}H_{27}NO_5$	325.18837
	MOD B_2	$C_{15}H_{23}NO_3$	265.16725

Table S2. Fragment ions of peptide (SLVYPFPGP^{80}) modified by ATZ2. Red and blue numbers indicate fragments that were matched with theoretical masses of corresponding fragments; black numbers indicate fragments not detected. Related to Figure 2C-D.

#1	b^+	b^{2+}	Seq.	y^+	y^{2+}	#2
1	88.03931	44.52329	S			9
2	201.12338	101.05533	L	1700.87809	850.94268	8
3	300.19180	150.59954	V	1587.79402	794.40065	7
4	463.25512	232.13120	Y	1488.72560	744.86644	6
5	560.30789	280.65758	P	1325.66228	663.33478	5
6	707.37631	354.19179	F	1228.60951	614.80839	4
7	804.42908	402.71818	P	1081.54109	541.27418	3
8	861.45055	431.22891	G	984.48832	492.74780	2
9			P-ATZ2	927.46685	464.23706	1

Table S3. Fragment ions of peptide (F^{67}AQTQSLVYPFPGPIPN) modified by ART1. Red and blue numbers indicate fragments that were matched with theoretical masses of corresponding fragments; black numbers indicate fragments not detected. Related to Figure 2C-D.

#1	b^+	b^{2+}	Seq.	y^+	y^{2+}	#2
1	473.26407	237.13567	F-ART1			17
2	544.30119	272.65423	A	1728.89561	864.95144	16
3	672.35977	336.68352	Q	1657.85849	829.43288	15
4	773.40745	387.20736	T	1529.79991	765.40359	14
5	901.46603	451.23665	Q	1428.75223	714.87975	13

6	988.49806	494.75267	S	1300.69365	650.85046	12
7	1101.58213	551.29470	L	1213.66162	607.33445	11
8	1200.65055	600.82891	V	1100.57755	550.79241	10
9	1363.71387	682.36057	Y	1001.50913	501.25820	9
10	1460.76664	730.88696	P	838.44581	419.72654	8
11	1607.83506	804.42117	F	741.39304	371.20016	7
12	1704.88783	852.94755	P	594.32462	297.66595	6
13	1761.90930	881.45829	G	497.27185	249.13956	5
14	1858.96207	929.98467	P	440.25038	220.62883	4
15	1972.04614	986.52671	I	343.19761	172.10244	3
16	2069.09891	1035.05309	P	230.11354	115.56041	2
17			N	133.06077	67.03402	1

Table S4. HPLC purity and SMILES for the final compounds. Related to Figure 1A and 5A

ID	Purity (%)	SMILES
PI01	>95	O=C(NCCNC([C@H](CC(NC(C)(C)C)=O)NC(CCCNC(OC(C)(C)C)=O)=O)=O)C1=C(F)C=CC(C2=C(F)C=CC=C2)=C1
ART1	>95	C[C@]1(O[C@@]2([H])O[C@H](CC(N)=O)[C@@H]3C)CC[C@]4([H])[C@@]2(OO1)[C@@]3([H])CC[C@H]4C
ATZ1	>95	C[C@]1(O[C@@]2([H])O[C@H](CC(N[C@@H](CC(NC(C)(C)C)=O)C(NCCNC(C3=C(F)C=CC(C4=C(F)C=CC=C4)=C3)=O)=O)=O)[C@@H]5C)CC[C@]6([H])[C@@]2(OO1)[C@@]5([H])CC[C@H]6C
ATZ2	>95	C[C@]1(O[C@@]2([H])O[C@H](CC(NCC(N[C@@H](CC(NC(C)(C)C)=O)C(NCCNC(C3=C(F)C=CC(C4=C(F)C=CC=C4)=C3)=O)=O)=O)=O)[C@@H]5C)CC[C@]6([H])[C@@]2(OO1)[C@@]5([H])CC[C@H]6C
ATZ4	>95	O=C(NCCNC([C@@H](NC(CCCNC(C[C@H]([C@@H]1C)O[C@]2([H])O[C@@]3(C)CC[C@]4([H])[C@@]2(OO3)[C@@]1([H])CC[C@H]4C)=O)=O)CC(NC(C)(C)C)=O)=O)C5=C(F)C=CC(C6=C(F)C=CC=C6)=C5
Deoxy-ATZ4	>95	C[C@@](O[C@@]1([H])O[C@H](CC(NCCCC(N[C@@H](CC(NC(C)(C)C)=O)C(NCCNC(C2=C(F)C=CC(C3=C(F)C=CC=C3)=C2)=O)=O)=O)=O)[C@@H]4C)(O5)CC[C@]6([H])[C@@]15[C@@]4([H])CC[C@H]6C
ATZ-P1	>95	C[C@]1(O[C@@]2([H])O[C@H](CC(N[C@@H](CCCN=[N+]=[N-])C(N[C@@H](CC(NC(C)(C)C)=O)C(NCCNC(C3=C(F)C=CC(C4=C(F)C=CC=C4)=C3)=O)=O)=O)=O)[C@@H]5C)CC[C@]6([H])[C@@]2(OO1)[C@@]5([H])CC[C@H]6C
Deoxy-ATZ-P1	>95	C[C@@](O[C@@]1([H])O[C@H](CC(N[C@@H](CCCN=[N+]=[N-])C(N[C@@H](CC(NC(C)(C)C)=O)C(NCCNC(C2=C(F)C=CC(C3=C(F)C=

	CC=C3)=C2)=O)=O)=O)[C@@H]4C)(O5)CC[C@]6([H])[C@@]15[C@@]4([H])CC[C@H]6C