Table 1. Significant siRNA chemical modifications to address siRNA drawbacks.

siRNA modified moiety	siRNA chemical modification	Example	Functions/comments	Ref.
Sugar	2′-O-Me	ALN-VSP02 (ASC-06), ALN-HBV, ONPATTRO (Patisiran), ARO-HBV, ALN-HBV02, Atu027	Enhancing binding affinity, melting temperature (Tm), and nuclease stability; reducing immune activation	[1, 2]
	2′-O-MOE		This alteration commonly has been employed in the 3′-overhangs of siRNA (employed just in the sense strand); enhancing melting temperature (Tm) and nuclease stability; reducing immune activation	[3, 4]
	2′-F	ALN-HBV, ONPATTRO (Patisiran), ARO-HBV, ALN-HBV02	In every part of both sense and antisense strands can be partially modified, and there are studies of active siRNAs, which are completely changed with 2′F-RNA; enhancing binding affinity, melting temperature (Tm), and nuclease stability; reducing immune activation	[2]
	2′-O-cyanoethyl		Improving interaction affinity and nuclease resistance	[5]
	2′-O-acetalester		Can be employed to develop protected siRNA molecules	[6]
	2′-esterified units (levulinates)		Can be employed to develop protected siRNA molecules	[7]
	2′-O-DNP		Improving interaction affinity and resistance to nucleases while in some cases, somewhat reducing activity	[8]
	4′-S		Enhancing binding affinity and nuclease stability; is compatible with siRNA activity when placed close to the terminal of siRNA duplexes	[9, 10]
	Simultaneous application of 2′-O-MOE with 4′S-RNA and 2′-O-Me		Improving potency and serum stability	[11]
	2′F-ANA		Can be tolerated in completely modified sense strands and partially modified antisense strands siRNA; improving binding affinity and nuclease resistance	[12]
	4′S-2′F-ANA		Does not hinder siRNA activity at different positions in both strands. 2′F-ANA modifications in the sense strand are synergistic with 4′S-2′F-ANA in the antisense strand; limited modifications can be applied following the reduction of interaction affinity.	[13]
	LNA		Improving binding affinity to RNA, which results from conformational rigidity	[14]
	UNA		Reducing binding affinity to RNA	[15]

	tc-DNA		Can improve silencing activity when placed in the overhangs	[16]
	CeNA		Can improve the potency of siRNA	[17]
	ANAs		Can increase the potency and duration of silencing activity when placed at the proper position	[17]
	HNAs		Improving the potency of siRNA	[18]
	Morpholino		Can be employed in the sense strand and on the overhangs; can suppress silencing activity in the antisense strand; can eliminate backbone charges	[19]
Backbone Linkage Modifications	PS	ALN-VSP02 (ASC-06), ALN-HBV, ARO-HBV, ALN-HBV02	Enhancing nonspecific protein binding	[2]
	Amide-linked		Enhancing thermodynamic stability and nuclease resistance of siRNA duplex	[11]
	Phosphonoacetate		can eliminate backbone charges via esterification leading to cellular uptake without transfection reagent	[20]
	Phosphorothioate		Can increase potency of siRNA	[21]
	PNA		Enhancing thermodynamic stability, hydrophobicity, and nuclease resistance of siRNA duplex; can eliminate backbone charges	[22, 23]
	2′,5′-linked		Reducing the potency of siRNA	[24]
Base Modifications	5-Me-U		Enhancing siRNA stability and effective gene silencing by siRNA	[25]
	5-Me-C		Enhancing siRNA stability and effective gene silencing by siRNA	[25]
	GNA	ALN-HBV02, ALN-AGT	Improving thermal stability; enhancing siRNA stability against snake venom phosphodiesterase; increasing siRNA potencies	[2, 26]
	Diaminopurine		Can improve the strength of A-U base pairs	[27]
	2-thiouracil		Improving binding affinity, potency, and specificity of siRNA	[28]
	Pseudouracil		Can improve the strength of A-U base pairs	[28]
	2,4-difluorobenzene		Can be tolerated in specific positions of siRNA; in some cases, can increase the specificity of siRNA	[29]
	2,4-dichlorobenzene		Can be tolerated in specific positions of siRNA; in some cases, can increase the specificity of siRNA	[29]
Terminal Conjugates	Inverted abasic end cap	ARO-HBV, AMG 890, ARO-ANG3	Can improve exonuclease stability; can be used in biophysical/biochemical studies as a result of biotin or fluorescent dyes conjugation	[2]

Cholesterol conjugated	Can protect siRNA duplex from HSV-2 after intravaginal administration	[30]

Abbreviation: 2′-O-Me, 2′-methoxy group substitution; 2′-F, 2′- fluoro substitution; 2′-O-DNP, 2′-O-dinitrophenyl ethers; 2′F-ANA, HNAs, hexitol nucleic acids; 2′-deoxy-2′- fluoroarabinonucleic acids; LNA, locked nucleic acid; UNA, unlocked nucleic acid; CeNA, cyclohexenyl nucleic acids; ANAs, altritol nucleic acids; PS, Phosphorothioate; PNA, peptide nucleic acid; GNA, glycol nucleic acid; 2′-O-MOE, 2′-O-methoxyethyl; tc-DNA, tricyclo-DNA modification

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