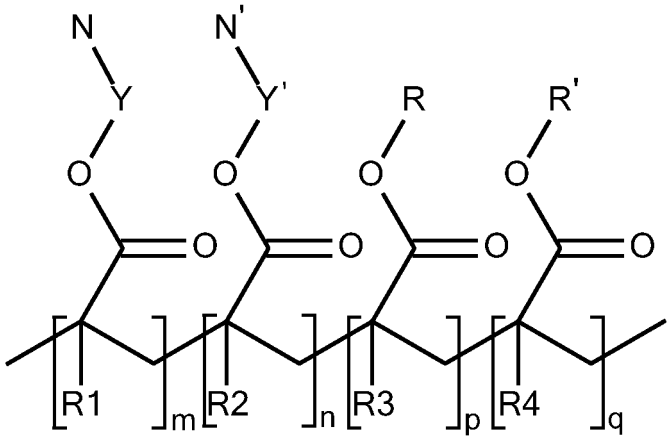
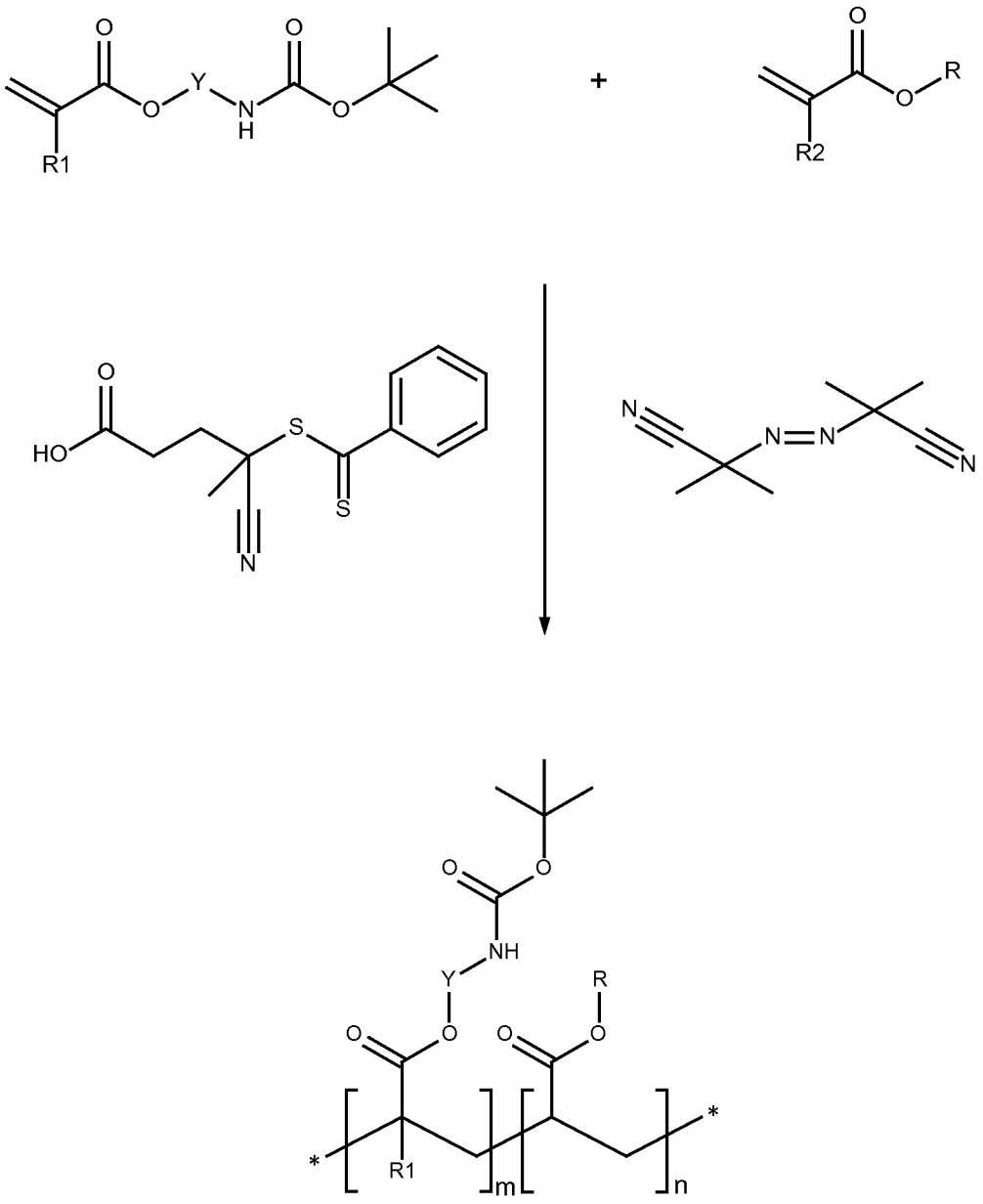


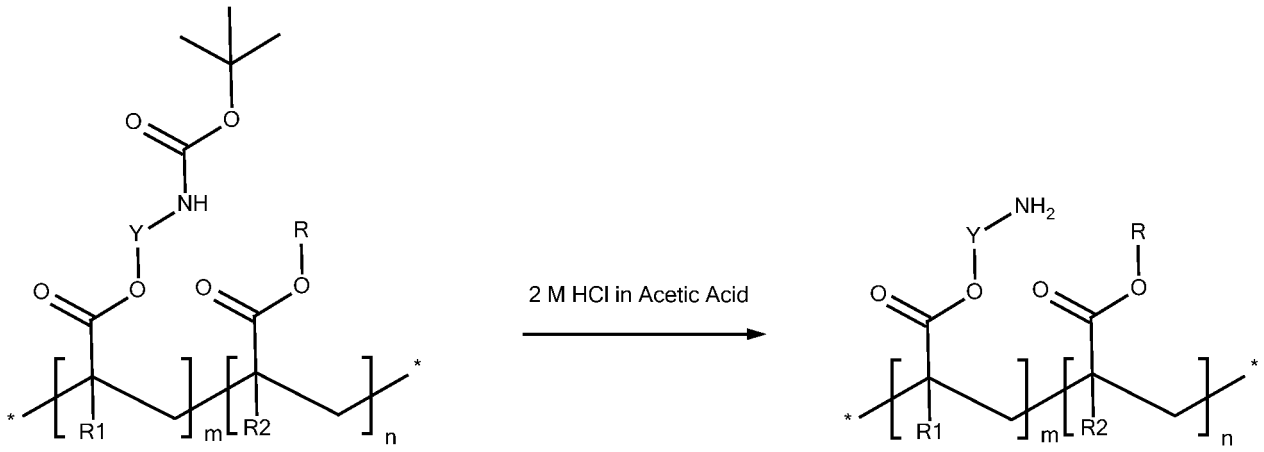
amphipathic poly(acrylate) random copolymer

FIG. 1



RAFT polymerization

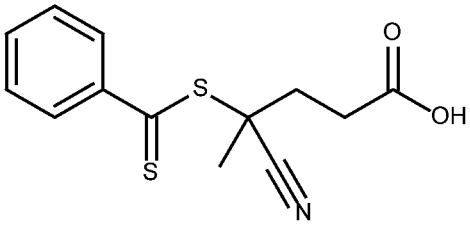
FIG. 2



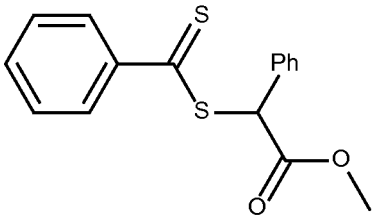
Amine Deprotection of Polymers

FIG. 3

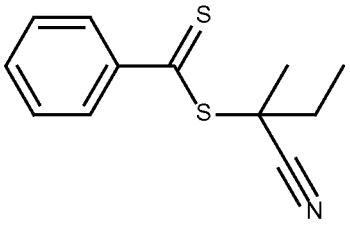
A.



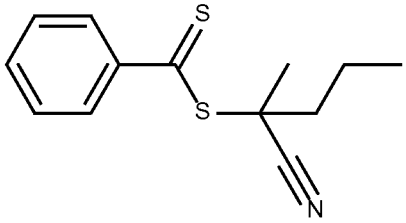
B.



C.

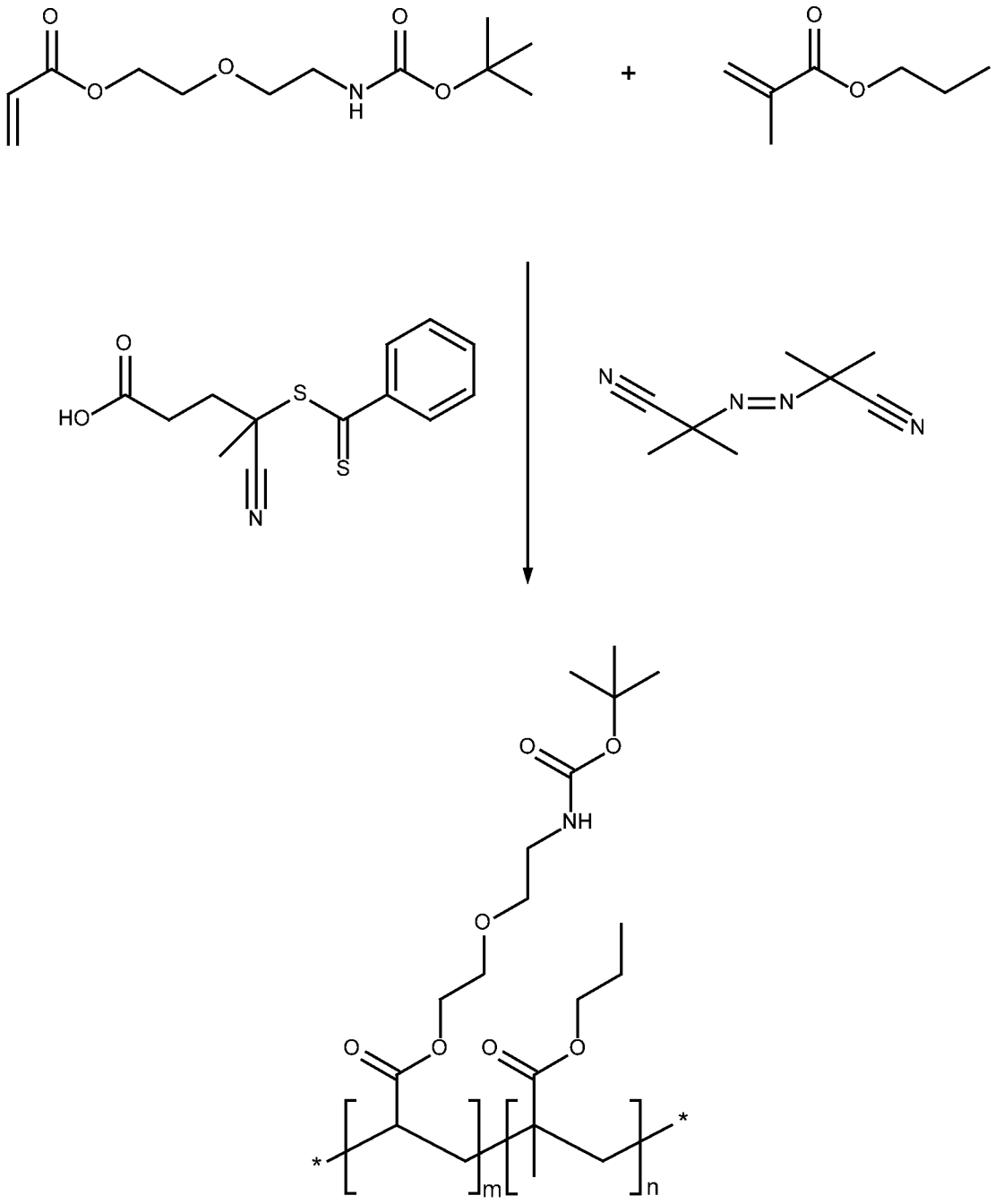


D.



exemplary RAFT Reagents

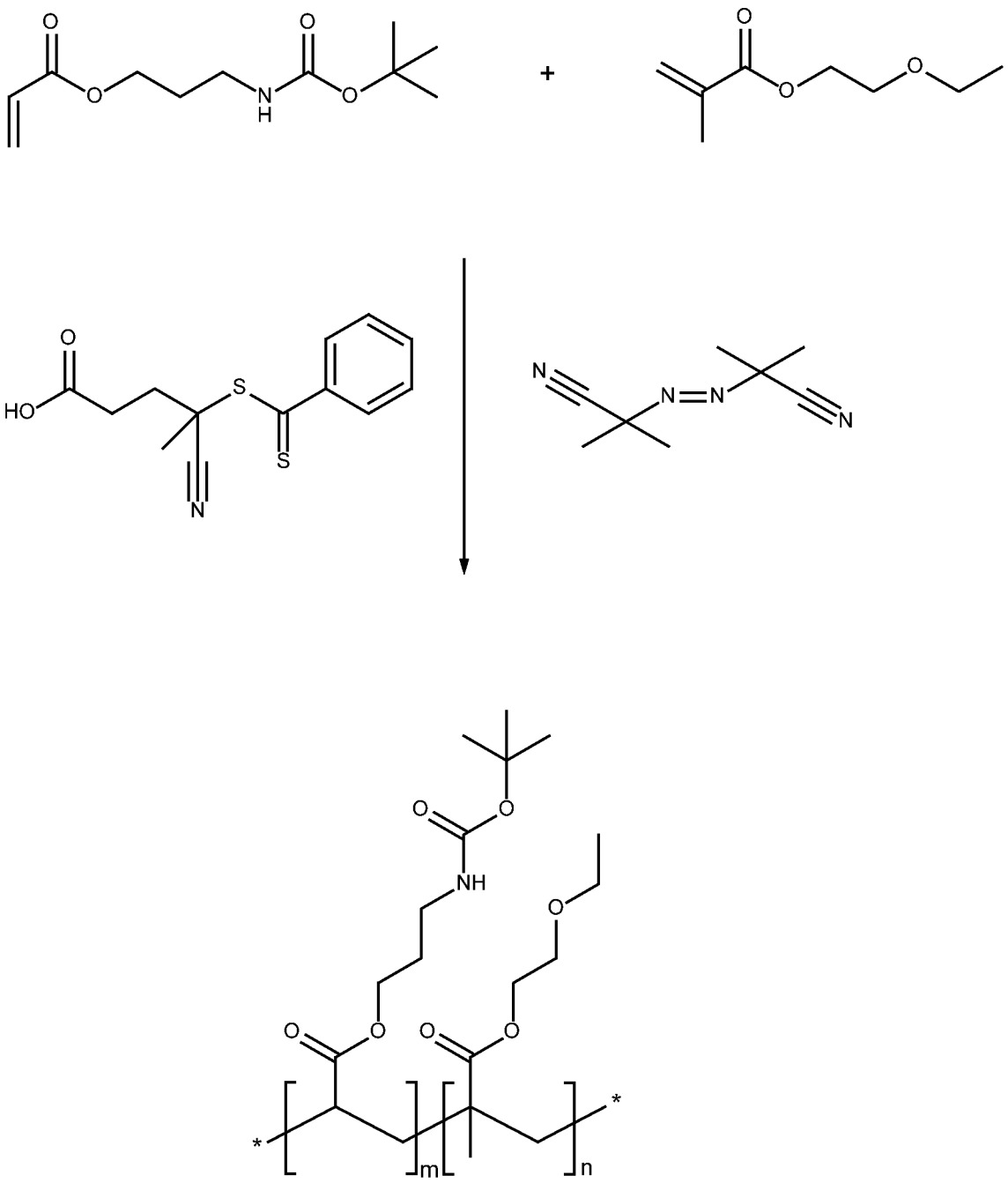
FIG. 4



amine protected LAU 41648-140-B-fr1

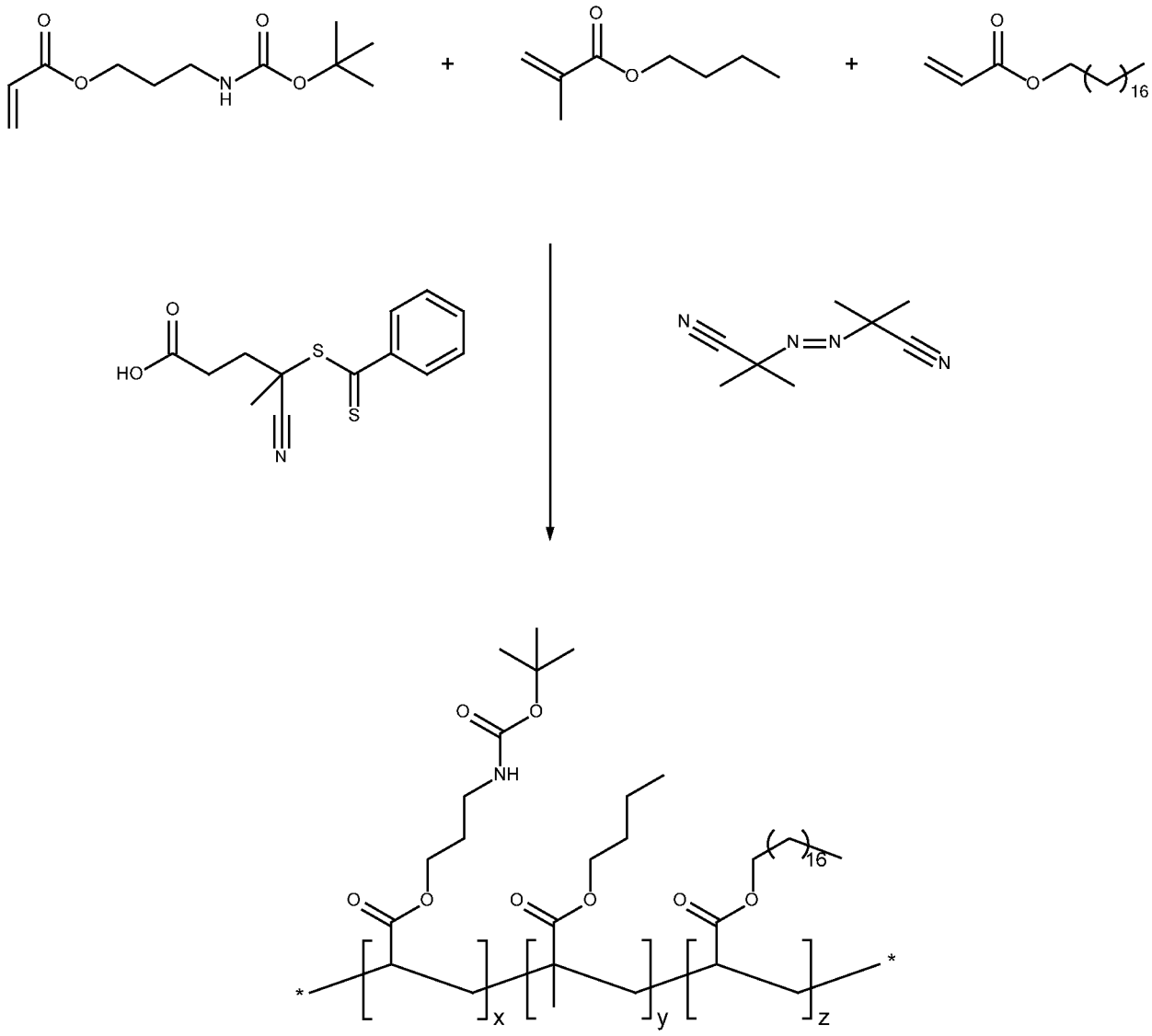
m = 72%, n = 28%

FIG. 5



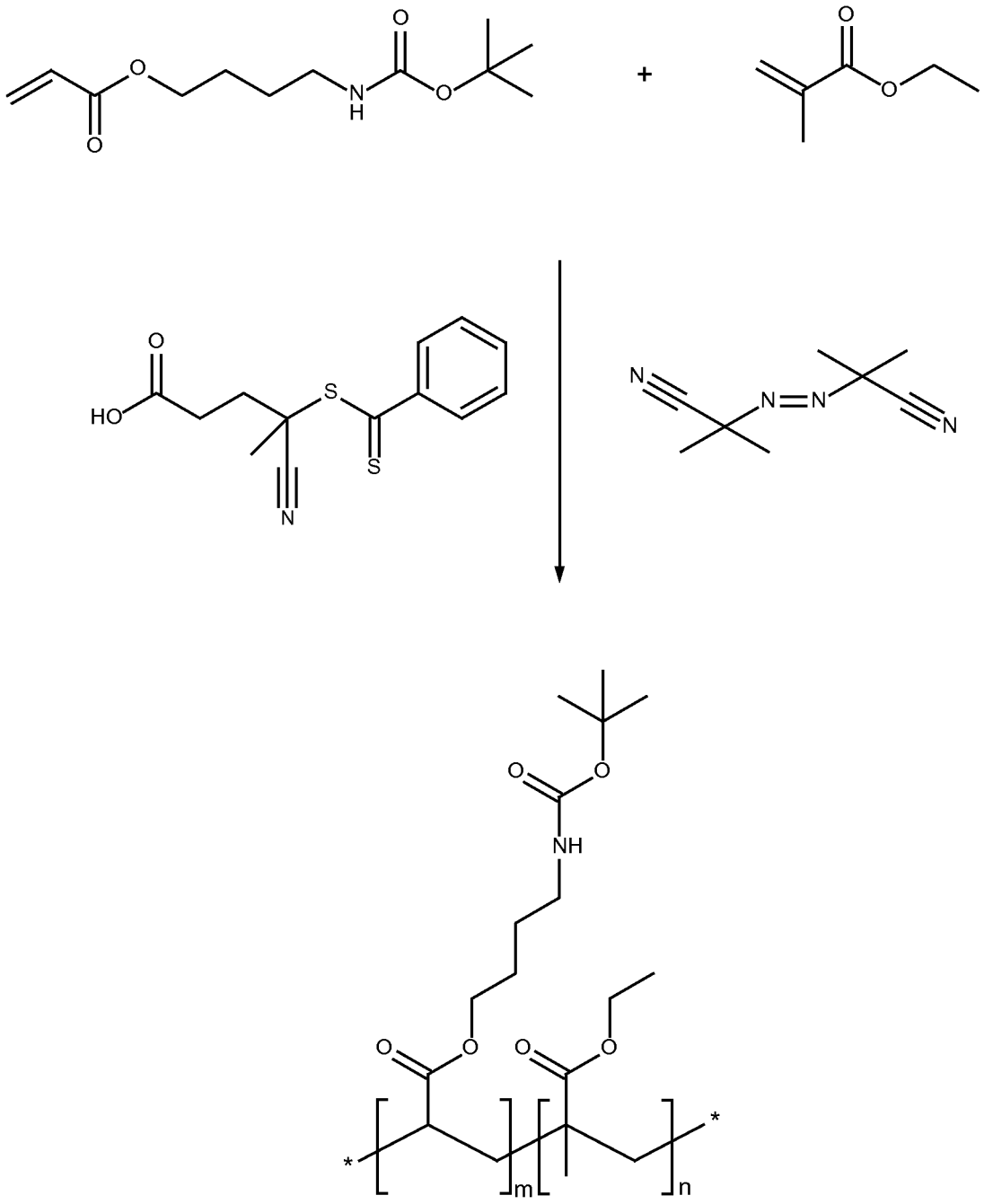
amine protected LAU 42101-23-D-fr1

FIG. 6



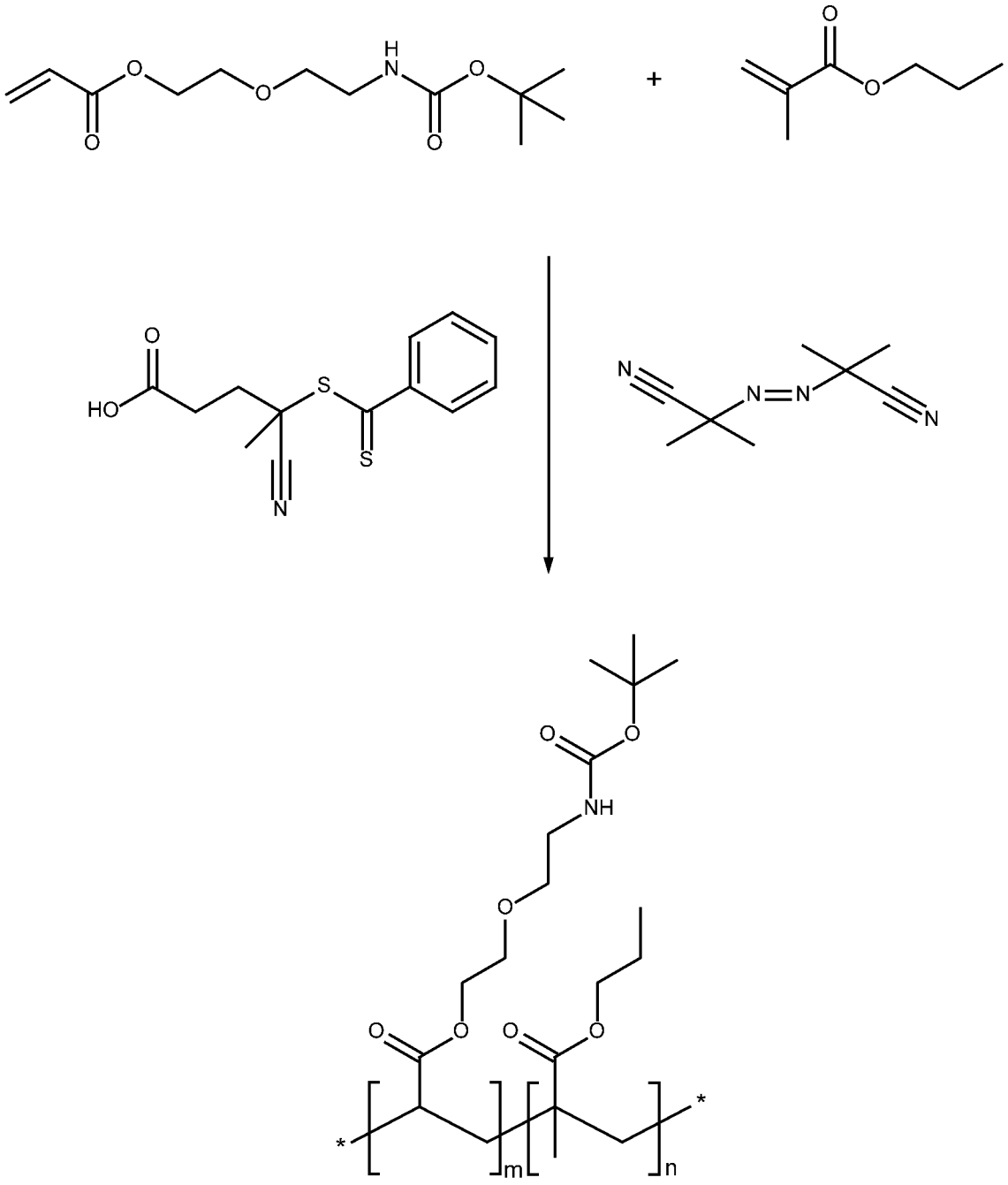
amine protected NAR 42020-117A-fr1

FIG. 7



amine protected NAR 41439-141B-fr1

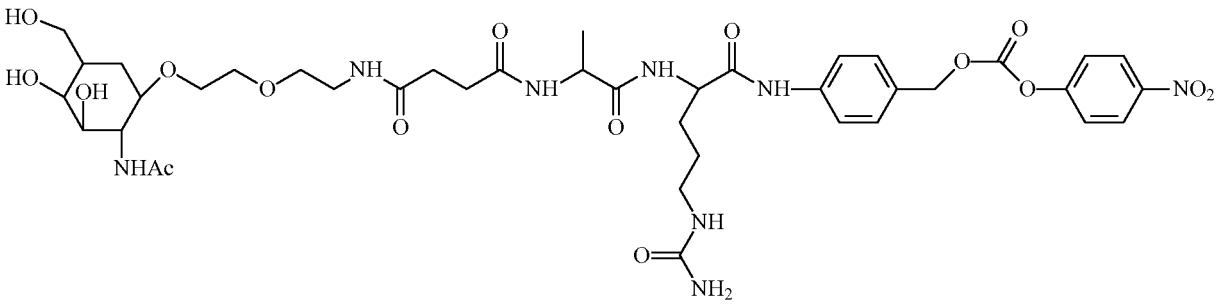
FIG. 8



amine protected NAR 41439-71B-fr1

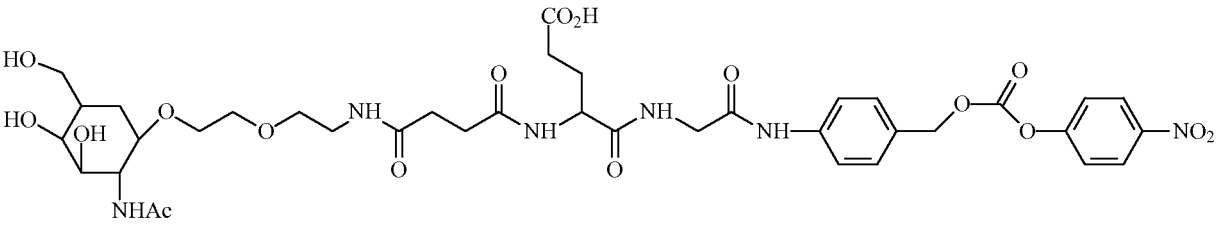
FIG. 9

A.



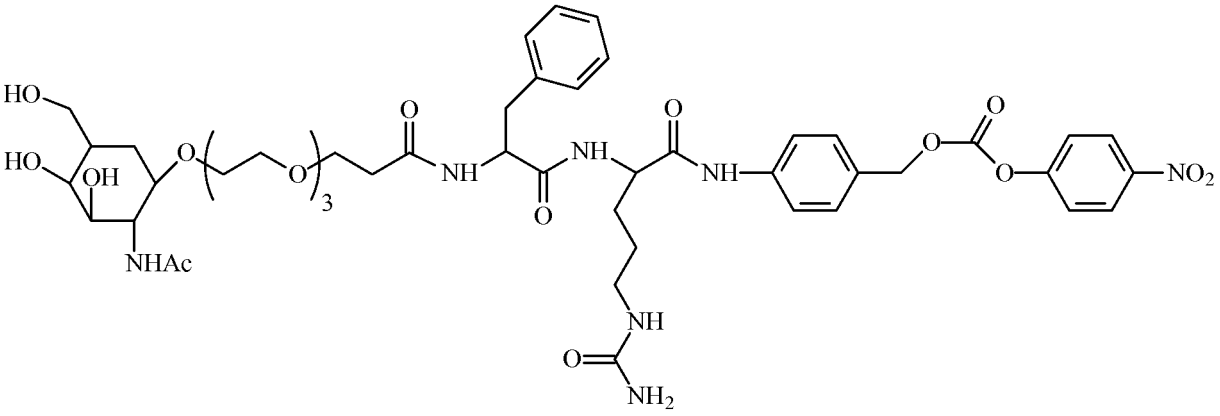
NAG-AlaCit-PABC-PNP

B.



NAG-GluGly-PABC-PNP

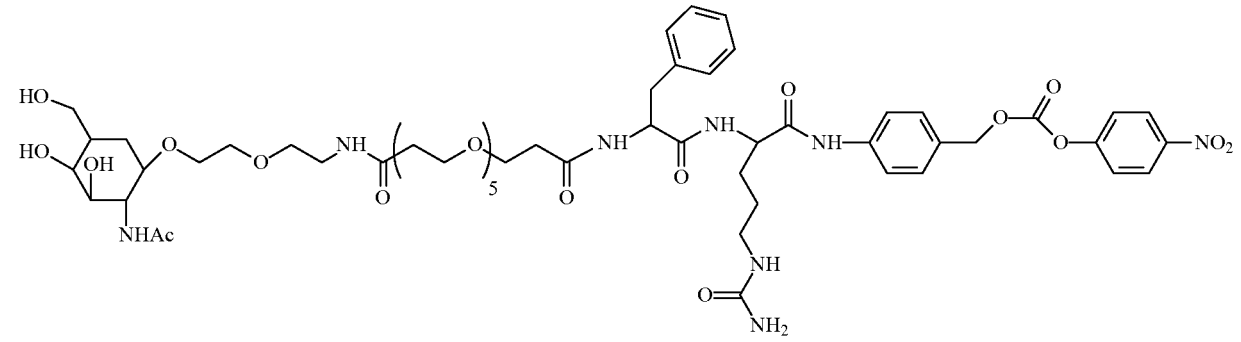
C.



NAG-PEG4-PheCit- PABC-PNP

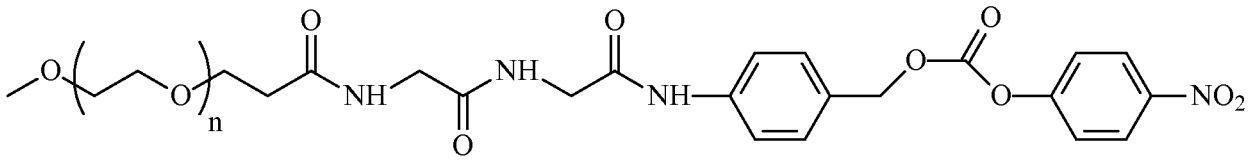
FIG. 10

D.



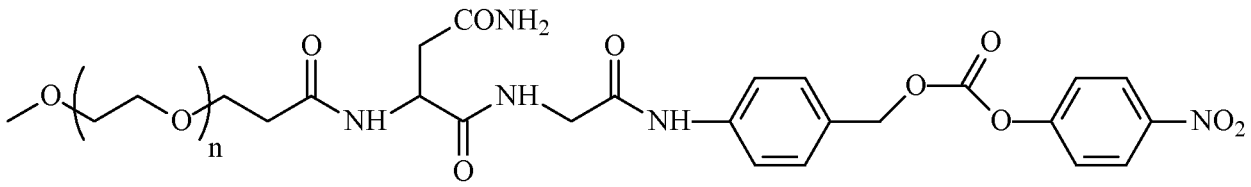
NAG-PEG7-PheCit-PABC-PNP

E.



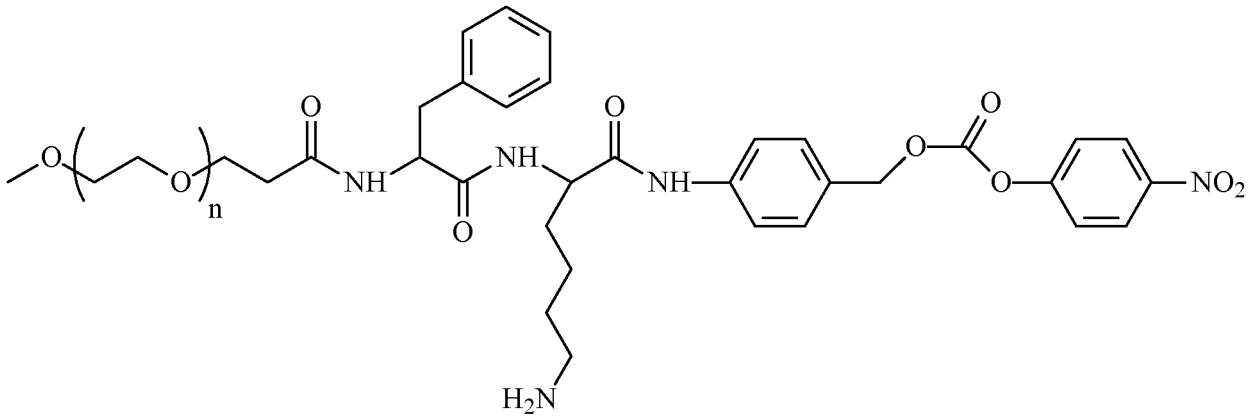
PEG-GlyGly-PABC-PNP

F.



PEG-AsnGly-PABC-PNP

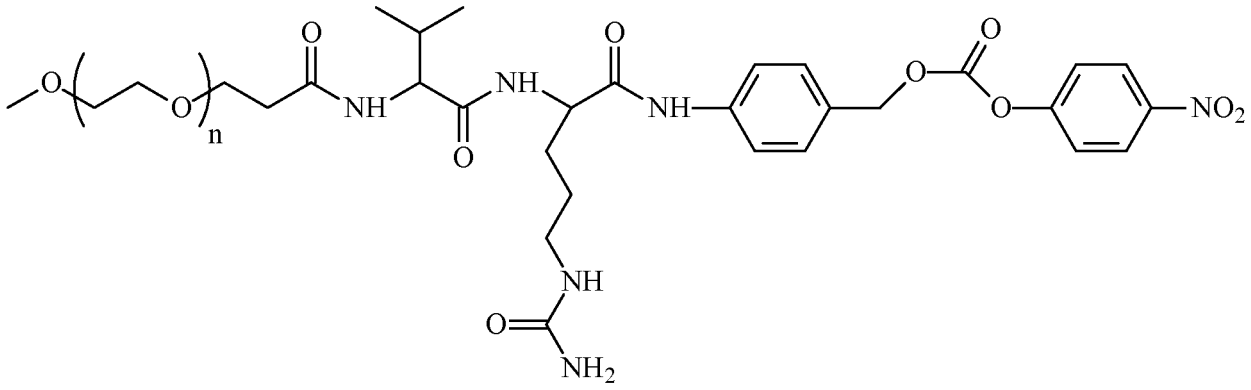
G.



PEG-PheLys-PABC-PNP

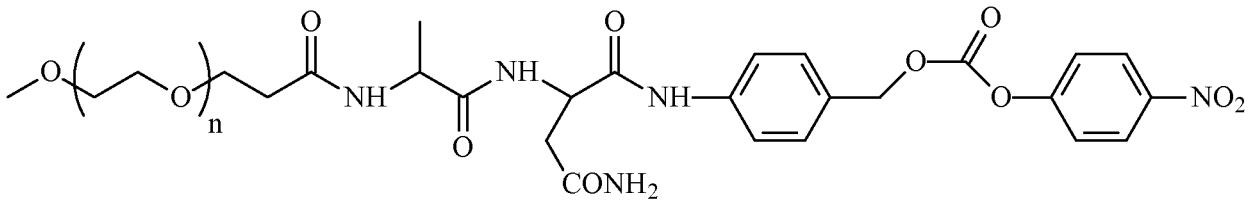
FIG. 10 (cont.)

H.



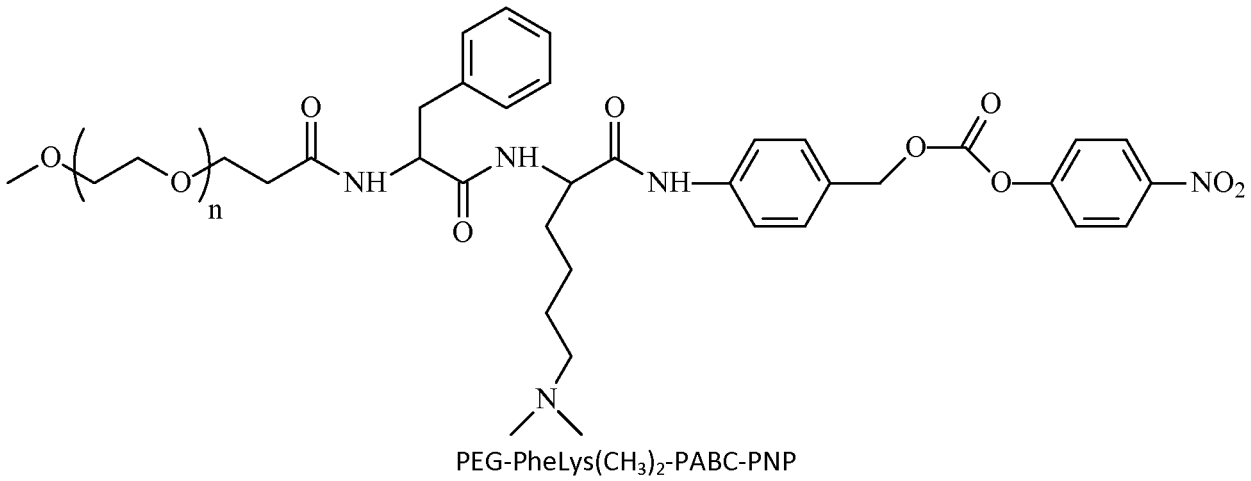
PEG-ValCit-PABC-PNP

I.



PEG-AlaAsn-PABC-PNP

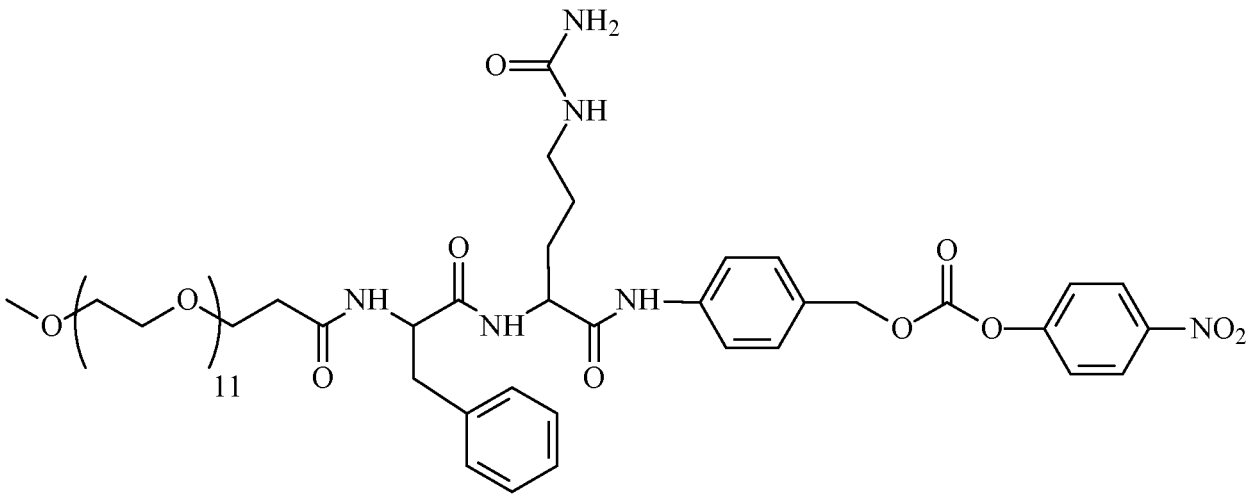
J.



PEG-PheLys$(CH_3)_2$-PABC-PNP

FIG. 10 (cont.)

K.



PEG_{12}-Phe-Cit-PABC-PNP

Dipeptide masking agents

FIG. 10 (cont.)

Percent Aha1 knockdown *in vitro* following delivery of 500 ng/mL Aha1 siRNA with 3.0 or 1.5 µg/ml ethylaminoacrylate + butyl methacrylate (EAA-BuMA) copolymers.

polymer		cell type									
		Hep3B-SEAP		MCF7		HT29		HepG2-SEAP		A375	
% Amine	MW	3.0 µg/ml	1.5 µg/ml	3.0 µg/ml	1.5 µg/ml	3.0 µg/ml	1.5 µg/ml	3.0 µg/ml	1.5 µg/ml	3.0 µg/ml	1.5 µg/ml
45%	54.4	87%	85%	87%	51%	87%	51%	81%	65%	89%	68%
46%	41.7	81%	85%	83%	54%	80%	59%	73%	72%	92%	81%
47%	29.9	79%	85%	60%	46%	50%	24%	69%	65%	48%	40%
58%	34.4	87%	90%	81%	83%	69%	72%	66%	52%	93%	91%
70%	27.3	82%	83%	51%	51%	25%	20%	56%	36%	87%	77%
72%	46.5	78%	81%	41%	41%	8%	19%	53%	31%	82%	63%
84%	25.6	47%	32%								
86%	47.5	32%	23%								

FIG. 11

Percent Aha1 knockdown *in vitro* following delivery of 500 ng/mL Aha1 siRNA with 3.0 or 1.5 µg/ml ethoxyethylaminoacrylate + *sec*-butyl acrylate (EEAA-SecBuA) copolymers.

polymer		cell type									
		Hep3B-SEAP		MCF7		HT29		HepG2-SEAP		A375	
% Amine	MW	3.0 µg/ml	1.5 µg/ml	3.0 µg/ml	1.5 µg/ml	3.0 µg/ml	1.5 µg/ml	3.0 µg/ml	1.5 µg/ml	3.0 µg/ml	1.5 µg/ml
53%	24.5	57%	65%	26%	23%			32%	28%	41%	31%
53%	40.5	57%	75%	21%	19%			41%	10%	64%	52%
53%	59.4	75%	85%	43%	38%			52%	31%	68%	74%
59%	24.8	55%	53%	24%	19%			31%	26%	39%	32%
58%	56.3	74%	78%	51%	29%			50%	21%	66%	70%
64%	24.0	43%	41%	24%	15%			25%	17%	29%	19%
64%	47.2	54%	57%	42%	26%			30%	20%	68%	65%
68%	24.5	29%	30%	17%	11%					17%	12%
69%	58.8	49%	49%	39%	21%					57%	57%

FIG. 12

Percent Aha1 knockdown *in vitro* following delivery of 500 ng/mL Aha1 siRNA with 3.0 or 1.5 µg/ml ethoxyethylaminoacrylate + butyl acrylate (EEAA-BuA) copolymers.

polymer		cell type									
		Hep3B-SEAP		MCF7		HT29		HepG2-SEAP		A375	
% Amine	MW	3.0 µg/ml	1.5 µg/ml	3.0 µg/ml	1.5 µg/ml	3.0 µg/ml	1.5 µg/ml	3.0 µg/ml	1.5 µg/ml	3.0 µg/ml	1.5 µg/ml
42%	30.2	81%	80%	56%	56%			71%	48%	74%	75%
42%	63.0	90%	87%	75%	68%			62%	44%	85%	72%
42%	87.6	89%	75%	64%	56%			59%	33%	81%	71%
53%	26.6	54%	79%	14%	20%			42%	27%	38%	40%
53%	53.2	72%	85%	34%	44%			45%	29%	78%	65%
53%	78.4	82%	86%	49%	54%			56%	38%	80%	79%
73%	47.1	44%	45%	22%	21%					41%	33%
73%	72.3	48%	46%	33%	17%					54%	43%

FIG. 13

Poly(acrylate) Random Copolymers: Composition.

polymer	amine monomer[j]	feed ratio (%)	actual ratio[b] (%)	hydrophobic monomer[i]	feed ratio (%)	actual ratio[b] (%)	Molecular weight kDa (×1000) theoretical[c]	Molecular weight kDa (×1000) calculated[d]	RAFT[a]	PDI[e]
Lau 42101-24ABC-1	ethoxyethyl	72.5	68	propyl meth	27.5	32	50	30	yes	1.1
Lau-41648-140-B fr1		72.5	66		27.5	34	50	21	yes	1.1
Lau-42101-24A-1		72.5	66		27.5	34	50	24	yes	1.1
Lau-42101-24B-1		72.5	69		27.5	31	50	25	yes	1.1
Ant 41658-111		75	n.d.		25	n.d.	73	52	yes	1.2
Ant 41658-111 fr1		75	n.d.		25	n.d.	87	62	yes	1.1
NAR 41439-71B-fr1		76	69		24	31	86	61	yes	1.5
Lau-41648-106 17-19		80	74		20	26		42	no	1.2
Lau 42101-43A-1	ethoxyethyl	80	74	ethoxyethyl meth	20	26	100	56	yes	1.2
Lau 42101-43B-1		75	70		25	30	100	53	yes	1.2
Lau 42101-43C-1		70	62		30	38	100	53	yes	1.2
Lau 42101-43D-1		65	57		35	43	100	56	yes	1.2
Lau 42101-44A-1	ethoxyethyl	80	75	butoxyethyl meth	20	25	100	60	yes	1.2
Lau 42101-44B-1		75	70		25	30	100	56	yes	1.2
Lau 42101-44C-1		70	64		30	36	100	60	yes	1.1
Lau 42101-44D-1		65	59		35	41	100	62	yes	1.1
Lau 1005-29A-1[f]	ethoxyethyl	55	61	sec-butyl	45	39	100	49	yes	1.1
Lau 42101-8D-1		55	59		45	41	100	47	yes	1.1

FIG. 14

Lau 1005-6C-1	ethoxyethyl	55	58	propyl	45	42	100	55	yes	1.1
Lau 1005-6D-1		50	53		50	47	100	56	yes	1.1
Lau 1005-12H-1	ethoxyethyl	50	53	butyl	50	47	150	79	yes	1.2
Lau 42101-13B-1	ethoxyethyl	65	66	iso-amyl	35	34	100	38	yes	1.3
Lau 41305-38 17-19	propyl	80	n.d.	ethyl meth	20	n.d.		62	no	1.4
Lau-41648-102 17-19		80	78		20	23		54	no	1.4
Nar-41439-116A fr1		76	70		24	30	79	56	yes	1.5
Ant 40911-123-3		80	74		20	26	86	52	no	1.3
Ant-41658-58 [g]	propyl	70	n.d.	butyl meth	25	n.d.	133	92	no	1.1
NAR 42020-117A-fr1 [h]		76	65		24	30	190	128	yes	1.1
Lau-42101-23D1	propyl	68	59	ethoxyethyl meth	32	41	50	39	yes	1.1
NAR 41439-141B-fr1	butyl	79	67	ethyl meth	21	33	49	34	yes	1.1

[a]- polymer synthesized by RAFT polymerization

[b]- amine content experimentally determined

[c]- theoretical molecular weight of protected polymer

[d]- molecular weight for deprotected polymer calculated from experimentally measured molecular weight for protected polymer

[e]- polydispersity measured for protected polymer

[f]- polymer also contained 0.001% 9-anthracenylmethyl acrylate feed ratio which is not expected to affect biological properties of the polymer

[g]- polymer also contained 5% octadecyl monomer feed ratio

[h]- polymer also contained 5% octadecyl monomer feed and actual ratios

[i]- corresponds to group **R** of formula (**I**) or (**Ia**), "meth" signifies methacrylate monomer

j- corresponds to spacer group **Y** of formula (**I**) or (**Ia**)

n.d. = not determined

FIG. 14 cont.

In vivo gene knockdown following injection of reversibly masked polymer conjugated to siRNA.

polymer		masking [b]		siRNA		species		injection		harvest (days)	% gene inhibition
	amount			gene	amount		n	site	vol.		
isotonic glucose [a]											0
Lau 42101-8D	250 μg	2x FC-PEG(24)	6x AC-NAG	Factor VII	20 μg	mouse	3	subQ [c]	300 μL	5	91
	500 μg	2x FC-PEG(24)	6x AC-NAG	Factor VII	50 μg	mouse	3	subQ	300 μL	5	99
	200 μg	2x FC-PEG(12)	6x AC-NAG	Factor VII	10 μg	mouse	3	IV	300 μL	2	96
	100 μg	4.7x CDM-PEG	2.3x CDM-NAG	Factor VII	10 μg	mouse	3	IV	300 μL	2	94
	250 μg (1 mg/kg)	4.7x CDM-PEG	2.3x CDM-NAG	Factor VII	50 μg	rat	3	IV	1000 μL	2	93
Lau-42101-24A-1	250 μg	4.7x CDM-PEG	2.3x CDM-NAG	Factor VII	50 μg	rat	3	IV	1000 μL	2	91
	2500 μg	4.7x CDM-PEG	2.3x CDM-NAG	Factor VII	50 μg	rat	3	IV	1000 μL	2	100
	250 μg	2x FC-PEG(24)	6x FC-NAG	Factor VII	50 μg	rat	3	IV	1000 μL	2	15
	2500 μg	2x FC-PEG(24)	6x FC-NAG	Factor VII	50 μg	rat	3	IV	1000 μL	2	99
	250 μg	2x FC-PEG(24)	6x FC-NAG	Factor VII	50 μg	rat	3	IV	1000 μL	2	42
	250 μg	2x FC-PEG(24)	5x CDM-NAG	Factor VII	50 μg	rat	3	IV	1000 μL	2	55
	250 μg	2x AN-PEG	5x CDM-NAG	Factor VII	50 μg	rat	3	IV	1000 μL	2	40
	250 μg	2x VC-PEG	5x CDM-NAG	Factor VII	50 μg	rat	3	IV	1000 μL	2	43
Lau-42101-23D-1-fr1	150 μg	4.7x CDM-PEG	2.3x CDM-NAG	Factor VII	40 μg	mouse		IV	400 μL	2	95

FIG. 15

Lau 41648-140-B	500 μg	2x FC-PEG(24)	6x AC-NAG	Factor VII	50 μg	mouse	3	subQ	300 μl	5	99
	100 μg	2x FC-PEG(12)	6x AC-NAG	Factor VII	10 μg	mouse	3	IV	300 μL	3	52
	300 μg	4.7x CDM-PEG	2.3x CDM-NAG	ApoB	10 μg	mouse	3	IV	200 μl	3	50
	5 mg/kg	4.7x CDM-PEG	2.3x CDM-NAG	*control*	1 mg/kg	primate		IV	2 mL/kg	8	7 [d]
	0.5 mg/kg	4.7x CDM-PEG	2.3x CDM-NAG	Factor VII	0.1 mg/kg	primate		IV	2 mL/kg	8	80
	1 mg/kg	4.7x CDM-PEG	2.3x CDM-NAG	Factor VII	0.2 mg/kg	primate		IV	2 mL/kg	8	94
	2.5 mg/kg	4.7x CDM-PEG	2.3x CDM-NAG	Factor VII	0.5 mg/kg	primate		IV	2 mL/kg	8	90
Lau 29A-1	5000 μg (20 mg/kg)	2x FC-PEG(12)	6x AC-NAG	Factor VII	250 μg	rat	3	subQ	500 μL	7	59
	750 μg	2x FC-PEG(12)	6x AC-NAG	Factor VII	50 μg	rat	3	IV	1000 μL	10	72
Ant-41658-111	500 μg	4.7x CDM-PEG	2.3x CDM-NAG	Factor VII	50 μg	mouse	3	subQ	300 μL	5	51

[a]- isotonic glucose control

[b]- numbers represent of mg masking agent per mg polymer

[c]- subQ signifies subcutaneous injection

[d]- compared to day 1

FIG. 15 cont.

In vivo gene knockdown following co-injection of reversibly masked polymer and cholesterol-siRNA.

polymer		masking [b]		siRNA		species		injection		harvest (days)	% gene inhibition
	amount			gene	amount		n	site	vol. (μL)		
isotonic glucose [a]											0
Ant-41658-111	150 μg	4.7x CDM-PEG	2.3x CDM-NAG	ApoB	40 μg	mouse	3	IV	200 μL	2	86
Lau 42101-8D	150 μg	4.7x CDM-PEG	2.3x CDM-NAG	ApoB	40 μg	mouse	3	IV	200 μL	2	82
	500 μg	2x FC-PEG(24)	6x AC-NAG	Factor VII	100 μg	mouse	3	subQ [c]	300 μL	5	80
	300 μL	2x FC-PEG(12)	6x AC-NAG	Factor VII	100 μg	mouse	3	IV	300 μL	2	99
Ant 123-3	1250 μg	4.7x CDM-PEG	2.3x CDM-NAG	Factor VII	750 μg	rat		IV	1000 μL	2	85

[a]- isotonic glucose control

[b]- numbers represent of mg masking agent per mg polymer

[c]- subQ signifies subcutaneous injection

FIG. 16