Effects of Polycation End-group Functionalization on Plasmid DNA Uptake and Transfection

Kevin Anderson[†], Antons Sizovs[§], Mallory Cortez[†], Chris Waldron, David M. Haddleton, Theresa M. Reineke[†]*

[†]Department of Chemistry, University of Minnesota, Minneapolis, MN 55455

[§]Department of Chemistry, Virginia Tech, Blacksburg, VA 24061

Department of Chemistry, University of Warwick, CV4 7AL, Coventry, UK

*To Whom Correspondence Should be Addressed: treineke@umn.edu

Supporting Information

DMEM		<i>p</i> -value
Oligoethyleneamine Tr4 N/P 15	luc	< 0.0001
Oligoethyleneamine Tr4 N/P 15	Azido Trehalose Tr4 N/P 15	< 0.0001
Oligoethyleneamine Tr4 N/P 15	Carboxy Tr4 N/P 15	< 0.0001
Oligoethyleneamine Tr4 N/P 15	Azido Trehalose Tr4 N/P 7	< 0.0001
Oligoethyleneamine Tr4 N/P 15	Adamantane Tr4 N/P 15	< 0.0001
Oligoethyleneamine Tr4 N/P 15	GLYCO	< 0.0001
Oligoethyleneamine Tr4 N/P 15	Alkynyl-oligoethyleneamine Tr4 N/P 15	< 0.0001
Oligoethyleneamine Tr4 N/P 15	Octyl Tr4 N/P 7	< 0.0001
Oligoethyleneamine Tr4 N/P 15	Alkynyl-oligoethyleneamine Tr4 N/P 7	< 0.0001
Oligoethyleneamine Tr4 N/P 15	Oligoethyleneamine Tr4 N/P 7	< 0.0001
Oligoethyleneamine Tr4 N/P 15	Adamantane Tr4 N/P 7	< 0.0001
Oligoethyleneamine Tr4 N/P 15	PEI	< 0.0001
Oligoethyleneamine Tr4 N/P 15	Carboxy Tr4 N/P 7	< 0.0001
Oligoethyleneamine Tr4 N/P 15	Octyl Tr4 N/P 15	< 0.0001
Octyl Tr4 N/P 15	luc	0.0008
Octyl Tr4 N/P 15	Azido Trehalose Tr4 N/P 15	0.0035
Carboxy Tr4 N/P 7	luc	0.0046
Octyl Tr4 N/P 15	Carboxy Tr4 N/P 15	0.0105
Octyl Tr4 N/P 15	Azido Trehalose Tr4 N/P 7	0.0124
Octyl Tr4 N/P 15	Adamantane Tr4 N/P 15	0.0133
Carboxy Tr4 N/P 7	Azido Trehalose Tr4 N/P 15	0.0194
Octyl Tr4 N/P 15	GLYCO	0.0262
Octyl Tr4 N/P 15	Alkynyl-oligoethyleneamine Tr4 N/P 15	0.0530
Carboxy Tr4 N/P 7	Carboxy Tr4 N/P 15	0.0538
PEI	luc	0.0606
Carboxy Tr4 N/P 7	Azido Trehalose Tr4 N/P 7	0.0624
Carboxy Tr4 N/P 7	Adamantane Tr4 N/P 15	0.0663
Octyl Tr4 N/P 15	Octyl Tr4 N/P 7	0.1091
Carboxy Tr4 N/P 7	GLYCO	0.1196
Octyl Tr4 N/P 15	Alkynyl-oligoethyleneamine Tr4 N/P 7	0.1220
PEI	Azido Trehalose Tr4 N/P 15	0.1951
Carboxy Tr4 N/P 7	Alkynyl-oligoethyleneamine Tr4 N/P 15	0.2146
Adamantane Tr4 N/P 7	luc	0.2210
Octyl Tr4 N/P 15	Oligoethyleneamine Tr4 N/P 7	0.2388
Carboxy Tr4 N/P 7	Octyl Tr4 N/P 7	0.3723
PEI	Carboxy Tr4 N/P 15	0.4004
Carboxy Tr4 N/P 7	Alkynyl-oligoethyleneamine Tr4 N/P 7	0.4034
PEI	Azido Trehalose Tr4 N/P 7	0.4392
PEI	Adamantane Tr4 N/P 15	0.4557
Adamantane Tr4 N/P 7	Azido Trehalose Tr4 N/P 15	0.5244
Oligoethyleneamine Tr4 N/P 7	luc	0.5453
Octyl Tr4 N/P 15	Adamantane Tr4 N/P 7	0.5733
Carboxy Tr4 N/P 7	Oligoethyleneamine Tr4 N/P 7	0.6240
PEI	GLYCO	0.6317
Alkynyl-oligoethyleneamine Tr4 N/P 7	luc	0.7647
Adamantane Tr4 N/P 7	Carboxy Tr4 N/P 15	0.7912
Octyl Tr4 N/P 7	luc	0.7947
PEI	Alkynyl-oligoethyleneamine Tr4 N/P 15	0.8104

DMEM		<i>p</i> -value
Adamantane Tr4 N/P 7	Azido Trehalose Tr4 N/P 7	0.8252
Adamantane Tr4 N/P 7	Adamantane Tr4 N/P 15	0.8384
Oligoethyleneamine Tr4 N/P 7	Azido Trehalose Tr4 N/P 15	0.8717
Octyl Tr4 N/P 15	PEI	0.9137
Carboxy Tr4 N/P 7	Adamantane Tr4 N/P 7	0.9272
Alkynyl-oligoethyleneamine Tr4 N/P 15	luc	0.9319
PEI	Octyl Tr4 N/P 7	0.9400
Adamantane Tr4 N/P 7	GLYCO	0.9405
PEI	Alkynyl-oligoethyleneamine Tr4 N/P 7	0.9531
Alkynyl-oligoethyleneamine Tr4 N/P 7	Azido Trehalose Tr4 N/P 15	0.9712
Octyl Tr4 N/P 7	Azido Trehalose Tr4 N/P 15	0.9787
Oligoethyleneamine Tr4 N/P 7	Carboxy Tr4 N/P 15	0.9817
GLYCO	luc	0.9858
Oligoethyleneamine Tr4 N/P 7	Azido Trehalose Tr4 N/P 7	0.9879
Adamantane Tr4 N/P 7	Alkynyl-oligoethyleneamine Tr4 N/P 15	0.9884
Oligoethyleneamine Tr4 N/P 7	Adamantane Tr4 N/P 15	0.9899
PEI	Oligoethyleneamine Tr4 N/P 7	0.9944
Alkynyl-oligoethyleneamine Tr4 N/P 15	Azido Trehalose Tr4 N/P 15	0.9981
Adamantane Tr4 N/P 15	luc	0.9983
Azido Trehalose Tr4 N/P 7	luc	0.9987
Alkynyl-oligoethyleneamine Tr4 N/P 7	Carboxy Tr4 N/P 15	0.9988
Carboxy Tr4 N/P 7	PEI	0.9989
Oligoethyleneamine Tr4 N/P 7	GLYCO	0.9990
Adamantane Tr4 N/P 7	Octvl Tr4 N/P 7	0.9993
Octvl Tr4 N/P 7	Carboxy Tr4 N/P 15	0.9993
Carboxy Tr4 N/P 15	luc	0.9993
Alkynyl-oligoethyleneamine Tr4 N/P 7	Azido Trehalose Tr4 N/P 7	0.9994
Alkynyl-oligoethyleneamine Tr4 N/P 7	Adamantane Tr4 N/P 15	0.9996
Adamantane Tr4 N/P 7	Alkynyl-oligoethyleneamine Tr4 N/P 7	0.9996
Octvl Tr4 N/P 7	Azido Trehalose Tr4 N/P 7	0.9997
Octyl Tr4 N/P 7	Adamantane Tr4 N/P 15	0.9998
GLYCO	Azido Trehalose Tr4 N/P 15	0.9999
Oligoethyleneamine Tr4 N/P 7	Alkynyl-oligoethyleneamine Tr4 N/P 15	0.9999
Alkynyl-oligoethyleneamine Tr4 N/P 7	GLYCO	0.9999
Octyl Tr4 N/P 15	Carboxy Tr4 N/P 7	0.9999
Alkynyl-oligoethyleneamine Tr4 N/P 15	Carboxy Tr4 N/P 15	0.9999
PEI	Adamantane Tr4 N/P 7	0.9999
Octvl Tr4 N/P 7	GLYCO	0.9999
Alkynyl-oligoethyleneamine Tr4 N/P 15	Azido Trehalose Tr4 N/P 7	0.9999
Adamantane Tr4 N/P 7	Oligoethyleneamine Tr4 N/P 7	0.9999
Alkynyl-oligoethyleneamine Tr4 N/P 15	Adamantane Tr4 N/P 15	0.9999
Azido Trehalose Tr4 N/P 15	luc	0.9999
Adamantane Tr4 N/P 15	Azido Trehalose Tr4 N/P 15	0.9999
Azido Trehalose Tr4 N/P 7	Azido Trehalose Tr4 N/P 15	0.9999
Carboxy Tr4 N/P 15	Azido Trehalose Tr4 N/P 15	0.9999
Oligoethyleneamine Tr4 N/P 7	Octvl Tr4 N/P 7	0.9999
Alkynyl-oligoethyleneamine Tr4 N/P 7	Alkynyl-oligoethyleneamine Tr4 N/P 15	1
GLYCO	Carboxy Tr4 N/P 15	1
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DMEM		<i>p</i> -value
Oligoethyleneamine Tr4 N/P 7	Alkynyl-oligoethyleneamine Tr4 N/P 7	1
Octyl Tr4 N/P 7	Alkynyl-oligoethyleneamine Tr4 N/P 15	1
GLYCO	Azido Trehalose Tr4 N/P 7	1
Alkynyl-oligoethyleneamine Tr4 N/P 15	GLYCO	1
GLYCO	Adamantane Tr4 N/P 15	-
Adamantane Tr4 N/P 15	Carboxy Tr4 N/P 15	-
Azido Trehalose Tr4 N/P 7	Carboxy Tr4 N/P 15	-
Alkynyl-oligoethyleneamine Tr4 N/P 7	Octyl Tr4 N/P 7	-
Adamantane Tr4 N/P 15	Azido Trehalose Tr4 N/P 7	_

Figure S1. Statistical analysis of the luciferase transfection data in DMEM containing 10% FBS (in Figure 7A in the main manuscript). ANOVA was conducted with Tukey-Kramer HSD analysis to compare the means of each relative light unit measurement. A *p*-value < 0.05 indicates a statistically significant difference between sample pairs of polyplexes displayed in each column and row.

Opti-MEM		<i>p</i> -value
PEI	Luc	0.0058
PEI	GLYCO	0.0075
PEI	Carboxy Tr4 N/P 7	0.0503
Adamantane Tr4 N/P 15	Luc	0.0547
Adamantane Tr4 N/P 15	GLYCO	0.0681
Oligoethyleneamine Tr4 N/P 15	Luc	0.0714
PEI	Oligoethyleneamine Tr4 N/P 7	0.0823
Oligoethyleneamine Tr4 N/P 15	GLYCO	0.0883
PEI	Azido Trehalose Tr4 N/P 15	0.0955
PEI	Alkynyl-oligoethyleneamine Tr4 N/P 7	0.1240
Azido Trehalose Tr4 N/P 7	Luc	0.1341
Azido Trehalose Tr4 N/P 7	GLYCO	0.1627
Octyl Tr4 N/P 15	Luc	0.3059
Adamantane Tr4 N/P 15	Carboxy Tr4 N/P 7	0.3115
Octyl Tr4 N/P 15	GLYCO	0.3571
Alkynyl-oligoethyleneamine Tr4 N/P 15	Luc	0.3689
Oligoethyleneamine Tr4 N/P 15	Carboxy Tr4 N/P 7	0.3752
PEI	Adamantane Tr4 N/P 7	0.3812
Alkynyl-oligoethyleneamine Tr4 N/P 15	GLYCO	0.4254
Adamantane Tr4 N/P 15	Oligoethyleneamine Tr4 N/P 7	0.4346
PEI	Octvl Tr4 N/P 7	0.4448
Adamantane Tr4 N/P 15	Azido Trehalose Tr4 N/P 15	0.4771
Oligoethyleneamine Tr4 N/P 15	Oligoethyleneamine Tr4 N/P 7	0.5086
Oligoethyleneamine Tr4 N/P 15	Azido Trehalose Tr4 N/P 15	0.5529
Adamantane Tr4 N/P 15	Alkynyl-oligoethyleneamine Tr4 N/P 7	0.5569
Azido Trehalose Tr4 N/P 7	Carboxy Tr4 N/P 7	0.5582
PEI	Carboxy Tr4 N/P 15	0.5975
Carboxy Tr4 N/P 15	luc	0.6284
Oligoethyleneamine Tr4 N/P 15	Alkynyl-oligoethyleneamine Tr4 N/P 7	0.6339
Carboxy Tr4 N/P 15	GLYCO	0.6904
Azido Trehalose Tr4 N/P 7	Oligoethyleneamine Tr4 N/P 7	0.6995
Azido Trehalose Tr4 N/P 7	Azido Trehalose Tr4 N/P 15	0.7412
Octyl Tr4 N/P 7	luc	0.7749
Azido Trehalose Tr4 N/P 7	Alkynyl-oligoethyleneamine Tr4 N/P 7	0.8107
Octyl Tr4 N/P 7	GLYCO	0.8266
Octyl Tr4 N/P 15	Carboxy Tr4 N/P 7	0.8281
Adamantane Tr4 N/P 7	luc	0.8316
PEI	Alkynyl-oligoethyleneamine Tr4 N/P 15	0.8421
Adamantane Tr4 N/P 7	GLYCO	0.8758
Alkynyl-oligoethyleneamine Tr4 N/P 15	Carboxy Tr4 N/P 7	0.8816
PEI	Octyl Tr4 N/P 15	0.8929
Adamantane Tr4 N/P 15	Adamantane Tr4 N/P 7	0.9038
Octyl Tr4 N/P 15	Oligoethyleneamine Tr4 N/P 7	0.9185
Adamantane Tr4 N/P 15	Octyl Tr4 N/P 7	0.9375
Octyl Tr4 N/P 15	Azido Trehalose Tr4 N/P 15	0.9387
Oligoethyleneamine Tr4 N/P 15	Adamantane Tr4 N/P 7	0.9405
Alkynyl-oligoethyleneamine Tr4 N/P 15	Oligoethyleneamine Tr4 N/P 7	0.9510
Oligoethyleneamine Tr4 N/P 15	Octyl Tr4 N/P 7	0.9643

Opti-MEM		<i>p</i> -value
Alkynyl-oligoethyleneamine Tr4 N/P 15	Azido Trehalose Tr4 N/P 15	0.9650
Octyl Tr4 N/P 15	Alkynyl-oligoethyleneamine Tr4 N/P 7	0.9661
Adamantane Tr4 N/P 15	Carboxy Tr4 N/P 15	0.9817
Alkynyl-oligoethyleneamine Tr4 N/P 15	Alkynyl-oligoethyleneamine Tr4 N/P 7	0.9826
Carboxy Tr4 N/P 15	Carboxy Tr4 N/P 7	0.9829
Azido Trehalose Tr4 N/P 7	Adamantane Tr4 N/P 7	0.9882
PEI	Azido Trehalose Tr4 N/P 7	0.9884
Alkynyl-oligoethyleneamine Tr4 N/P 7	luc	0.9911
Oligoethyleneamine Tr4 N/P 15	Carboxy Tr4 N/P 15	0.9916
Azido Trehalose Tr4 N/P 7	Octyl Tr4 N/P 7	0.9946
Alkynyl-oligoethyleneamine Tr4 N/P 7	GLYCO	0.9957
Carboxy Tr4 N/P 15	Oligoethyleneamine Tr4 N/P 7	0.9965
Azido Trehalose Tr4 N/P 15	luc	0.9966
Octyl Tr4 N/P 7	Carboxy Tr4 N/P 7	0.9970
Carboxy Tr4 N/P 15	Azido Trehalose Tr4 N/P 15	0.9981
Oligoethyleneamine Tr4 N/P 7	luc	0.9982
Azido Trehalose Tr4 N/P 15	GLYCO	0.9986
Adamantane Tr4 N/P 7	Carboxy Tr4 N/P 7	0.9989
PEI	Oligoethyleneamine Tr4 N/P 15	0.9990
Oligoethyleneamine Tr4 N/P 7	GLYCO	0.9993
Azido Trehalose Tr4 N/P 7	Carboxy Tr4 N/P 15	0.9994
Adamantane Tr4 N/P 15	Alkynyl-oligoethyleneamine Tr4 N/P 15	0.9994
Carboxy Tr4 N/P 15	Alkynyl-oligoethyleneamine Tr4 N/P 7	0.9995
Octyl Tr4 N/P 7	Oligoethyleneamine Tr4 N/P 7	0.9997
PEI	Adamantane Tr4 N/P 15	0.9997
Octyl Tr4 N/P 15	Adamantane Tr4 N/P 7	0.9998
Carboxy Tr4 N/P 7	luc	0.9998
Adamantane Tr4 N/P 15	Octyl Tr4 N/P 15	0.9999
Oligoethyleneamine Tr4 N/P 15	Alkynyl-oligoethyleneamine Tr4 N/P 15	0.9999
Octyl Tr4 N/P 7	Azido Trehalose Tr4 N/P 15	0.9999
Adamantane Tr4 N/P 7	Oligoethyleneamine Tr4 N/P 7	0.9999
Carboxy Tr4 N/P 7	GLYCO	1
Octyl Tr4 N/P 15	Octyl Tr4 N/P 7	1
Alkynyl-oligoethyleneamine Tr4 N/P 15	Adamantane Tr4 N/P 7	1
Adamantane Tr4 N/P 7	Azido Trehalose Tr4 N/P 15	1
Oligoethyleneamine Tr4 N/P 15	Octyl Tr4 N/P 15	1
Octyl Tr4 N/P 7	Alkynyl-oligoethyleneamine Tr4 N/P 7	1
Alkynyl-oligoethyleneamine Tr4 N/P 15	Octyl Tr4 N/P 7	1
Adamantane Tr4 N/P 7	Alkynyl-oligoethyleneamine Tr4 N/P 7	1
Azido Trehalose Tr4 N/P 7	Alkynyl-oligoethyleneamine Tr4 N/P 15	1
Octyl Tr4 N/P 15	Carboxy Tr4 N/P 15	1
Azido Trehalose Tr4 N/P 7	Octyl Tr4 N/P 15	1
Alkynyl-oligoethyleneamine Tr4 N/P 15	Carboxy Tr4 N/P 15	1
Adamantane Tr4 N/P 15	Azido Trehalose Tr4 N/P 7	1
Alkynyl-oligoethyleneamine Tr4 N/P 7	Carboxy Tr4 N/P 7	1
Carboxy Tr4 N/P 15	Adamantane Tr4 N/P 7	1
Oligoethyleneamine Tr4 N/P 15	Azido Trehalose Tr4 N/P 7	1
Azido Trehalose Tr4 N/P 15	Carboxy Tr4 N/P 7	1

Opti-MEM		<i>p</i> -value
Carboxy Tr4 N/P 15	Octyl Tr4 N/P 7	-
Oligoethyleneamine Tr4 N/P 7	Carboxy Tr4 N/P 7	-
Alkynyl-oligoethyleneamine Tr4 N/P 7	Oligoethyleneamine Tr4 N/P 7	-
Alkynyl-oligoethyleneamine Tr4 N/P 7	Azido Trehalose Tr4 N/P 15	-
Adamantane Tr4 N/P 15	Oligoethyleneamine Tr4 N/P 15	-
Octyl Tr4 N/P 15	Alkynyl-oligoethyleneamine Tr4 N/P 15	-
Octyl Tr4 N/P 7	Adamantane Tr4 N/P 7	-
GLYCO	luc	-
Azido Trehalose Tr4 N/P 15	Oligoethyleneamine Tr4 N/P 7	_

Figure S2. Statistical analysis of the luciferase transfection data in serum-free Opti-MEM (in Figure 7B in the main manuscript). ANOVA was conducted with Tukey-Kramer HSD analysis to compare the means of each relative light unit measurement. A *p*-value < 0.05 indicates a statistically significant difference between sample pairs of polyplexes displayed in each column and row.



Figure S3. GPC chromatogram of adamantane-Tr4 (1A) injected into 100 mM Na₂SO₄ in 1% acetic acid. A measured dn/dc value of 0.0935 ($r^2 = 0.999$) was applied. A solvent peak at 25 min is visible by dRI detection.



Figure S4. GPC chromatogram of carboxy-Tr4 (1B) injected into 100 mM Na₂SO₄ in 1% acetic acid. A measured dn/dc value of 0.0884 ($r^2 = 0.999$) was applied. A solvent peak at 25 min is visible by dRI detection.



Figure S5. GPC chromatogram of alkynyl oligoethyleneamine-Tr4 (1C) injected into 100 mM Na₂SO₄ in 1% acetic acid. A measured dn/dc value of 0.0565 ($r^2 = 0.996$) was applied. A solvent peak at 25 min is visible by dRI detection.



Figure S6. GPC chromatogram of azido trehalose-Tr4 (1D) injected into 100 mM Na₂SO₄ in 1% acetic acid. A measured dn/dc value of 0.0836 ($r^2 = 0.998$) was applied. A solvent peak at 25 min is visible by dRI detection.

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Figure S7. GPC chromatogram of octyl-Tr4 (1E) injected into 100 mM Na₂SO₄ in 1% acetic acid. A measured dn/dc value of 0.1179 ($r^2 = 0.995$) was applied. A solvent peak at 25 min is visible by dRI detection.



Figure S8. GPC chromatogram of oligoethyleneamine-Tr4 (1F) injected into 100 mM Na₂SO₄ in 1% acetic acid. A measured dn/dc value of 0.1216 ($r^2 = 0.999$) was applied. A solvent peak at 25 min is visible by dRI detection.



Figure S9. MALDI-TOF spectra of adamantane-Tr4 (1A) with analysis in linear mode. Sample and α -cyano-4-hydroxy cinnamic acid matrix were prepared in 50:50 acetonitrile : water.

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Figure S10. MALDI-TOF spectra of adamantane-Tr4 (1A) with analysis in reflectron mode. Molecular weight end functionalized with 1.5 repeating units: 1419.68 Da. Sample and 2,5-dihydroxybenzoic acid matrix were prepared in 50:50 acetonitrile : water with dilute sodium iodide.



Figure S11. MALDI-TOF spectra of carboxy-Tr4 (1B) with analysis in linear mode. Sample and α -cyano-4-hydroxy cinnamic acid matrix were prepared in 50:50 acetonitrile : water.



Figure S12. MALDI-TOF spectra of carboxy-Tr4 (1B) with analysis in reflectron mode. Molecular weight with 2 repeating units: 1457.51 Da. Sample and 2,5-dihydroxybenzoic acid matrix were prepared in 50:50 acetonitrile : water with dilute sodium iodide.



Figure S13. MALDI-TOF spectra of alkynyl oligoethyleneamine-Tr4 (1C) with analysis in linear mode. Sample and α -cyano-4-hydroxy cinnamic acid matrix were prepared in 50:50 acetonitrile : water.



Figure S14. MALDI-TOF spectra of alkynyl oligoethyleneamine-Tr4 (1C) with analysis in reflectron mode. Molecular weight with 2 repeating units: 1457.51 Da. Sample and 2,5-dihydroxybenzoic acid matrix were prepared in 50:50 acetonitrile : water with dilute sodium iodide.



Figure S15. MALDI-TOF spectra of azido trehalose-Tr4 (1D) with analysis in linear mode. Sample and α -cyano-4-hydroxy cinnamic acid matrix were prepared in 50:50 acetonitrile : water.



Figure 16. MALDI-TOF spectra of azido trehalose-Tr4 (1D) with analysis in reflectron mode. Molecular weight end-functionalized with 2.5 repeating units: 1849.83 Da. Sample and 2,5-dihydroxybenzoic acid matrix were prepared in 50:50 acetonitrile : water with dilute sodium iodide.



Figure S17. MALDI-TOF spectra of octyl-Tr4 (1E) with analysis in linear mode. Sample and α -cyano-4-hydroxy cinnamic acid matrix were prepared in 50:50 acetonitrile:water.



Figure S18. MALDI-TOF spectra of octyl-Tr4 (1E) with analysis in reflectron mode. Molecular weight end-functionalized with 2.5 repeating units: 2070.22 Da. Sample and 2,5-dihydroxybenzoic acid matrix were prepared in 50:50 acetonitrile : water with dilute sodium iodide.



Figure S19. MALDI-TOF spectra of oligoethyleneamine-Tr4 (1F) with analysis in linear mode. Sample and α -cyano-4-hydroxy cinnamic acid matrix were prepared in 50:50 acetonitrile : water.



Figure S20. MALDI-TOF spectra of oligoethyleneamine-Tr4 (1F) with analysis in reflectron mode. Molecular weight end-functionalized with 3.5 repeating units: 2418.63 Da. Sample and 2,5-dihydroxybenzoic acid matrix were prepared in 50:50 acetonitrile : water with dilute sodium iodide.