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Utilizing Electrostatic Interactions to Facilitate F-18 Radiolabeling of Poly(amido)amine (PAMAM) Dendrimers

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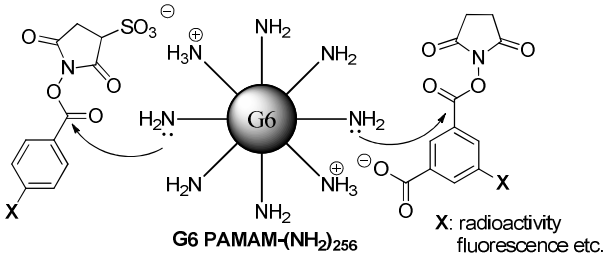

Electrostatic interactions facilitate conjugation reactions of cationic poly(amido)amine (PAMAM) dendrimers with anionic NHS reagents.

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Abstract

The development of methods for the facile conjugation and radiolabeling of poly(amido)amine (PAMAM) dendrimers would be of great benefit in evaluating biomedical applications of these intriguing molecularly defined polymers. Two anionic *N*-hydroxysuccinimide (NHS) esters (**7** and **10**) were developed and radiolabeled with fluorine-18 using Cu(I)-catalyzed click reactions. The radiolabeling of a primary amine-terminated PAMAM generation-6 (G6) dendrimer with [^{18}F]**7** or [^{18}F]**10** was complete in water or methanol within 5 min at room temperature. This highly efficient conjugation reaction benefits from a high, localized concentration of these NHS esters on the surface of PAMAM dendrimers, due to the electrostatic attraction between the anionic NHS esters and the positively-charged PAMAM dendrimers. The large medium effect (pH, salt, solvent) observed for these conjugation reactions is consistent with this mechanism. This novel strategy of utilizing electrostatic interactions provides a novel, facile, and efficient method for the conjugation and radiolabeling of PAMAM dendrimers that also has potential for radiolabeling other appropriate nanoparticles.

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Introduction

Poly(amido)amine) (PAMAM) dendrimers have been explored in a variety of biomedical applications, including as a platform for targeted delivery of therapeutic agents and for imaging purposes.[1-8] The functional groups on the surface of these structurally well-defined PAMAM dendrimers, especially in their whole integer generations (i.e., NH_2 groups), allow for functionalization of these dendrimers with multiple modalities. Among many reported strategies,[9-15] N-acylation with *N*-hydroxysuccinimide (NHS) esters is a commonly used method for conjugation reactions with amine-terminated PAMAM dendrimers.

PAMAM dendrimers have been labeled with different radioisotopes (^{3}H, ^{111}In, ^{88}Y, ^{177}Lu, ^{125}I, ^{99m}Tc, etc)[16-20] for biodistribution and pharmacokinetic studies, but up to now not with fluorine-18. Fluorine-18, in quantity and at high specific activity, is easily available from most medical cyclotrons, and radiolabeling of PAMAM dendrimers with ^{18}F would provide very useful tools for biomedical studies involving tracing the distribution of these interesting, molecularly defined polymers using positron emission tomographic (PET) imaging. In other work yet to be published, we have found that the distribution phase of ^{18}F labeled PAMAM dendrimers targeted to estrogen-responsive vascular tissues was complete within 2 hours, indicating that ^{18}F is an appropriate radiotracer for such species. However, radiolabeling of nanoparticles with ^{18}F, in general, is challenging, especially in terms of achieving high specific activity (SA), due to the short half-life of ^{18}F ($t_{1/2}$ = 109.8 min) and the generally slow kinetics of the labeling reaction under conventional conjugation conditions. Besides utilizing fast and specific coupling reactions,[12-15] another strategy to achieve fast kinetics is to have a mechanism for co-localizing the radiolabeling reagent with the target substrate.[21] PAMAM dendrimers of even integer generations are terminated in primary amines and are positively charged on their

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surface at physiological pH; this surface positive charge may enable a high concentration of negatively charged substances to become localized on their surface. In this paper, we report the facile conjugation and labeling reactions between cationic PAMAM dendrimers and anionic NHS reagents.

Results and discussion

Conjugation of PAMAM with NHS-fluorescein (1)

As a model for the coupling reaction of PAMAM G6 dendrimers, we first carried out a reaction with NHS-fluorescein (**1**) (see **Fig. 1**) in methanol, without addition of buffer or additional bases and using 4.6% or 46% molar equivalents of **1** relative to the 256 NH_2 groups on the periphery of this dendrimer. According to the HPLC analysis, within 5 min at room temperature the reaction with 4.6% of **1** was essentially complete and that with 46% molar equivalent of **1** was also nearly complete, with exclusive formation of the fluorescein conjugation product of the PAMAM (see supplementary data).

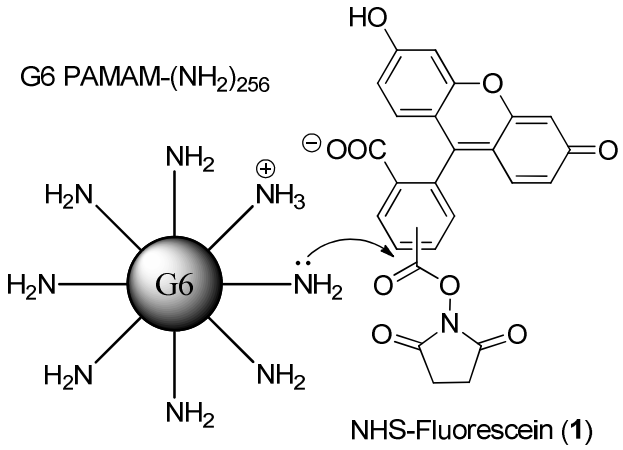

Figure 1. Conjugation of G6 PAMAM and NHS-fluorescein (**1**)

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Electrostatic uptake of fluorescein itself, which is anionic, into PAMAM dendrimers, which are cationic, has been reported.[22] Nevertheless, our further studies of the reaction between the PAMAM G6 dendrimer and fluorescein itself under the same conditions showed no coupling, indicating that covalent bond formation between PAMAM G6 and **1** had taken place in the above reaction. We presume that the initial reversible electrostatic uptake of anionic **1** on the cationic surface of the PAMAM G6 dendrimers results in a high, localized concentration of **1** on the dendrimer surface, driving the conjugation reaction of the NHS ester in **1** with NH_2 groups on the periphery of the dendrimer. This model reaction demonstrated the feasibility of fast, covalent radiolabeling of cationic PAMAM dendrimers with ^{18}F-labeled anionic NHS esters by capitalizing on the concentrating effect of these electrostatic interactions.

Synthesis and radiosynthesis of anionic NHS reagents

Two anionic NHS esters (**7** and **10**) were developed, as shown in **Scheme 1**. Compound **7** is a derivative of sulfo-NHS esters, which contains an anionic sulfonate group. Compound **10** is a derivative of an NHS ester that contains a second COOH group. Both [^{18}F]**7** and [^{18}F]**10** were radiosynthesized from the alkyne precursors **6** and **9**, respectively, using a Cu(I)-catalyzed click reaction with 2-[^{18}F]fluoroethyl azide, according to the methods described in our prior publication of the radiosynthesis of [^{18}F]**4**,[23] which is a NHS analogue of [^{18}F]**7**. A benzoic acid derivative [^{18}F]**5** was also generated from [^{18}F]**4** by hydrolysis.

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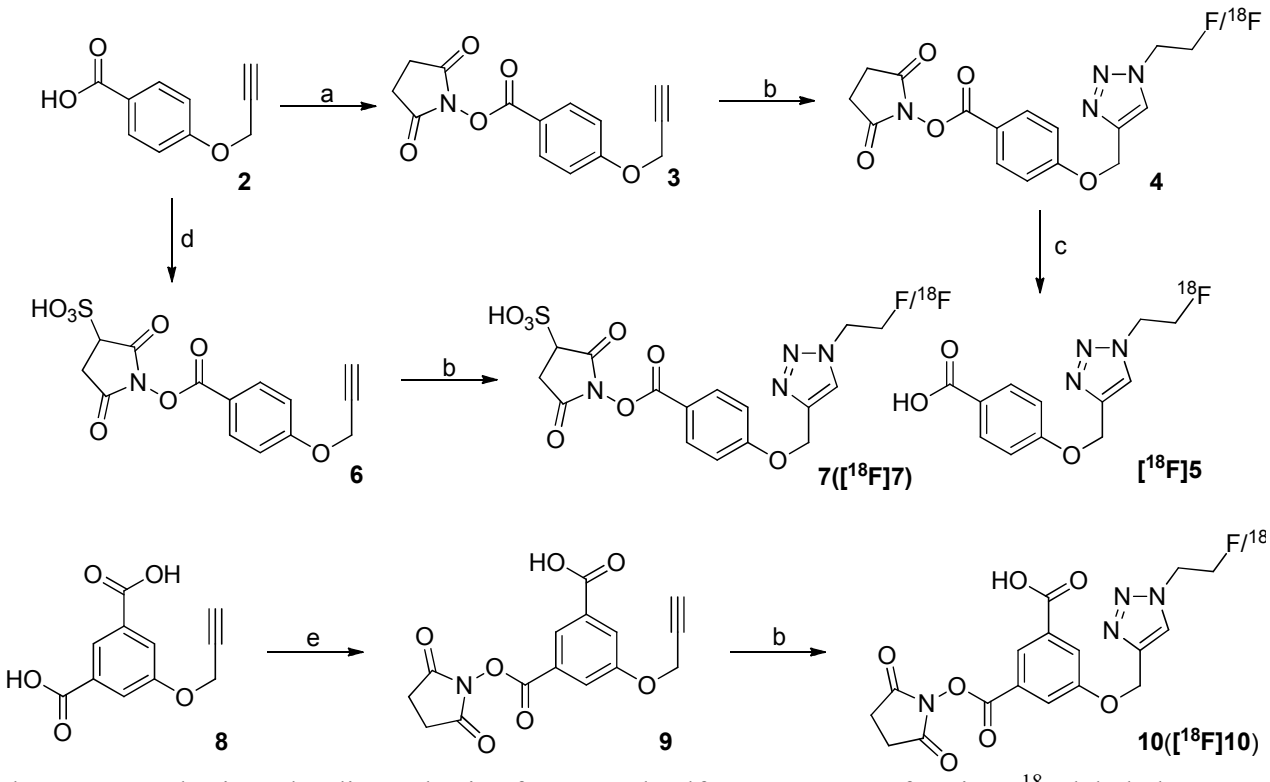

Scheme 1. Synthesis and radiosynthesis of NHS and sulfo-NHS esters of various ^{18}F-labeled benzoic acids.

Reaction conditions: (a) NHS-OH, DCC, DMF; (b) 2-fluoroethyl azide/2-[^{18}F]fluoroethyl azide, $CuSO_4$, Na ascorbate, BPDS, DMF; (c) NaOH; (d) sulfo-NHS-OH, DCC, DMF; (e) TSTU, Bu_4NOH, MeCN.

Radiolabeling of PAMAM with anionic NHS reagents

The conjugation reactions of PAMAM G6 with different ^{18}F-labeled reagents were carried out in a volume of 500 μL, which is a practical volume for radiolabeling. The radiochemical yields were determined by radio-TLC (see supplementary data). Analytical HPLC chromatographs of a conjugation of PAMAM G6 with [^{18}F]**7** and its purified product by Sephadex G-25 column is shown in the supplementary data. As shown in **Table 1**, the conjugation reactions with anionic NHS esters, [^{18}F]**7** and [^{18}F]**10**, were complete within 5 min at

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room temperature, whereas and in sharp contrast, the reactions with the neutral NHS ester [^{18}F]**4** and [^{18}F]SFB were slow. As expected, no reaction occurred with the simple anionic benzoic acid reagent [^{18}F]**5**. The difference in reaction rates between anionic and neutral NHS esters was also demonstrated by reactions with polyethylenimine (**Table 1**, note c). These results suggest that the fast conjugation reactions and covalent bond formation takes place as a result of the anionic NHS esters pre-localized on the surface of the cationic PAMAM dendrimers, due to the electrostatic attraction between the species of opposite charge.

Table 1. Summary of radiochemical yields (%) of PAMAM G6 with ^{18}F labeled substrates[a]

NHS	[^{18}F]**4**	[^{18}F]**5**	[^{18}F]**7**[c]	[^{18}F]**10**	[^{18}F]SFB[b,c]
5 min (%)	28.1±5.1	0	95.3±0.9	95.2±0.3	41±3.7
15 min (%)	71.3±9.6	0	93.5±0.9	96.3±0.3	76.9±1.2

Note: [a] G6 PAMAM 20 μg in 500 μL water, radiochemical yields were determined by radioTLC (silica TLC/MeOH), n = 3; [b] in 500 μL methanol, 2.1±0.2 % (5 min) and 5.0±2.0 % (15 min); [c] with 50 μg polyethylenimine (branched, average M_w ~25,000) in 500 μL methanol: 95.4±0.8 % (5 min), 96.1±0.5 % (15 min) for [^{18}F]**7** and 11.3±0.7 % (5 min), 24.6±2.5 % (15 min) for [^{18}F]SFB.

Table 2. Summary of radiochemical yields (%) with different amounts of PAMAM G6[a]

PAMAM (μg)	**400**		**40**		**4**		**0.4**	
Time (min)	5	15	5	15	5	15	5	15
[^{18}F]**7** (%)	92	92	92	92	90	90	17	31
[^{18}F]**10** (%)	91	91	91	91	91	91	8	18

Note: [a] Radiochemical yields were determined by radioTLC (silica TLC/MeOH), n = 1.

The high efficiency of this method was further probed by the reactions shown in **Table 2**. In these studies, the amount of PAMAM G6 in the labeling reactions was gradually reduced. Nevertheless, radiochemical yields in excess of 90% were achieved within 5 min with as low as 4 μg of PAMAM dendrimers in the 500-μL reaction volume. Only with 0.4 μg, which is an

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extremely low amount, was the reaction slow, but it still proceeded with time. The minimal amount of PAMAM dendrimers required for this type of coupling is ideal for producing radiolabeled dendrimers in high specific activity.

Medium effects

PAMAM dendrimers have a well-defined molecular architecture; pH and salt concentration, however, have great effects on the conformation of PAMAM dendrimers, and one can presume that they greatly affect the accessibility and reactivity of surface NH_2 groups: At low pHs, PAMAM dendrimers exhibit an open and extended conformation due to electrostatic repulsion between the dendrimer branches;[24] by contrast, at pH values higher than 9, the dendrimers adopt a compact structure that results from hydrogen bonds between branches.[25] High salt concentrations and solvent dielectric also have an impact on PAMAM dendrimer conformations and potentially also on dendrimer reactivity towards charged reagents, such as those we have been studying.[26] Therefore, we examined the effect of solvent and reaction medium on the conjugation reactions of PAMAMs with [^{18}F]**4** and [^{18}F]**7** (**Table 3**).

Table 3. Medium effects on conjugation yields (%) of PAMAM G6 with NHS and sulfo-NHS esters[a]

Solvent	Time	Water	0.1 % TFA	1 M NaCl	0.1 M $NaHCO_3$	MeOH	DMF
[^{18}F]**4**	5 min	28.1±5.1	0	5.4±0.5	6.1±0.3	0	0
	15 min	71.3±9.6	0	9.7±1.3	12.5±0.2	0	0
[^{18}F]**7**	5 min	95.2±0.5	0	26.8±1.4	11.1±0.2	93.1±0.9	0
	15 min	93.5±0.9	0	37.3±1.4	24.5±0.8	93.3±0.9	0

Note: [a] PAMAM G6 20 μg in 500 μL solvent, radiochemical yields were determined by radioTLC (silica TLC/MeOH), n = 3.

In a variety of solvents, the conjugation reactions with [^{18}F]**4** was slower than that with [^{18}F]**7**. Water was the ideal solvent for both [^{18}F]**4** and [^{18}F]**7**, but there was no reaction with the reagent, [^{18}F]**4**, in methanol, in sharp contrast to that with anionic [^{18}F]**7** in methanol. In 0.1%

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TFA, there was no reaction with either reagent: At pH 1, the NH_2 groups on the dendrimer would be fully protonated; the sulfonate group in [^{18}F]**7** would also be protonated, and being neutral, would no longer be undergo electrostatic concentration at the dendrimer surface, thereby interrupting the covalent reaction. Notably, when the labeling was conducted in 1 M aqueous NaCl, yields for both [^{18}F]**4** and [^{18}F]**7** were significantly reduced. Because the reactivity of both the neutral and anionic reagents is reduced, the salt effect is likely due to electrostatic screening that both reduces the local concentration of [^{18}F]**7** on the surface of the dendrimer and enables the PAMAM dendrimer to adopt a more compact morphology; the latter would reduce the reactivity of [^{18}F]**4** as well as [^{18}F]**7**. The conjugation reactions in 0.1 M aqueous $NaHCO_3$ proceeded very slowly, presumably because the dendrimer surface would be less positive at elevated pH. No reaction was observed in DMF. In brief, solvents and the pH and ionic strength of the medium have a great impact on the outcome of the conjugation reactions of PAMAM dendrimers.

Specific activity

The specific activity (SA) of radiolabeled PAMAM dendrimers is dependent on how many NHS substrates become conjugated on each dendrimer and the SA of radiolabeled reagent itself. In the above, diagnostic reactions, only about 1/30 to 1/50 of the final HPLC-purified products [^{18}F]**4** and [^{18}F]**7** were used for each reaction. The low stoichiometric ratio of ^{18}F-labeled reagent to PAMAM dendrimer in these cases would have a significant impact on the outcome of the conjugation reactions, ensuring high yields of activity incorporation but giving the final conjugated product in less than optimal SA because only a limited number of reagents would become attached to each dendrimer. Therefore, we examined these conjugation reactions using a large amount of [^{18}F]**4** and [^{18}F]**7**; under these conditions, the reagent [^{18}F]**7** still gave high

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labeling efficiency (94% at 5 min and 95% at 15 min), while the efficiency of labeling with [^{18}F]**4** was very much less (5% at 5 min and 13% at 15 min). When half of the final [^{18}F]**7** was used to conjugate with 20 μg PAMAM dendrimers in methanol, over 90% incorporation was achieved within 5 min; this afforded a product having two [^{18}F]**7** molecules conjugated per dendrimer, determined by comparing the SA of radiolabeled dendrimers with the SA of [^{18}F]**7**. In principle, the stoichiometric ratio of [^{18}F]**7** to PAMAM dendrimer could be increased so that multiple copies of the radiolabeled reagent per dendrimer are incorporated; this would result in a radiolabeled dendrimer having a SA multiple fold higher than that of the radiolabeled reagent itself.

PAMAM dendrimers provide an excellent platform for surface functionalization with a variety of moieties that are useful for many biomedical applications. However, using previously reported strategies, it is not easy to control the ligand/PAMAM ratio,[27] which is important for biological studies. The method reported here, utilizing electrostatic interaction to amplify reactivity, not only provides a facile method for radiolabeling, but also would enable efficient conjugation of other interesting ligands having biological activity in a process that allows for accurate control of the ligand/PAMAM ratios and easy purification. Because surface charge is common in nanoparticles, this strategy of harnessing electrostatic interactions to facilitate covalent labeling has the potential for improving the conjugation and labeling of many of these macromolecular species.

Experimental

All chemicals were obtained from standard commercial sources and used without further purification. All reactions were carried out by standard air-free and moisture-free techniques

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under an inert nitrogen atmosphere with dry solvents unless otherwise stated. Flash column chromatography was conducted using Scientific Adsorbents, Inc. silica gel, 60A, "40 Micron Flash" (32-63 μm). Routine ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz on an Agilent Technologies spectrometer. All chemical shifts were reported as parts per million (ppm) downfield from tetramethylsilane (TMS). All coupling constants (J) are given in Hertz (Hz). Splitting patterns are typically described as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Elemental analysis (C, H, N) were determined by Atlantic Microlab, Inc., Norcross, GA. High performance liquid chromatography (HPLC) was performed with an ultraviolet detector and a well-scintillation NaI (Tl) detector and associated electronics for radioactivity detection. An Agilent SB-C18 250 × 9.4 mm 5 μ semi-preparative column (A) and an Agilent SB-C18 250 × 4.6 mm 5 μ analytical column (B) were used for preparation and analysis respectively. [^{18}F]Fluoride was produced at Washington University by the $^{18}O(p,n)^{18}F$ reaction through proton irradiation of enriched (95%) [^{18}O] water in the RDS111 cyclotron. Radio-TLC was accomplished using a Bioscan AR-2000 imaging scanner (Bioscan, Inc., Washington, DC).

Synthesis of 5

A solution of 2-fluoroethyl azide (1.6 mmol) in DMF (3.5 mL) was added to a 10 mL screw-cap tube containing methyl 4-(prop-2-yn-1-yloxy)benzoate (0.2 g, 1.05 mmol), and followed by addition of Cu(I)/BPDS (100 μL from a mixture of 5 mg CuSO4•5H2O in 50 μL water, 15 mg sodium ascorbate in 50 μL water, and 6 mg BPDS 100 μL in 4:1 water/DMF). The reaction was stirred at room temperature for 2 h, and then at 50 ºC for 2 h to complete the reaction. The reaction mixture was diluted with water (50 mL), and then passed through a Waters Oasis® HLB cartridge (1 g), which was further rinse with water (3×10 mL). The product was eluted with methanol (7 mL), and NaOH (0.18 g, 4.5 mmol) in water (7 mL) was added to the above

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solution. The reaction mixture was stirred at room temperature for 24 h, and then was diluted with water (100 mL). The pH was adjusted to pH =1 using 1 N HCl solution. The formed white solids were filtered, rinsed with water (3×10 mL), and dried at room temperature to afford 5 (0.21 g) in 75% overall yield. 1H NMR (400 MHz, DMSO-d6) δ 12.64 (s, 1H), 8.27 (s, 1H), 7.87 (d, J = 8 Hz 2H), 7.10 (d, J = 8 Hz, 1H), 5.21 (s, 2H), 4.80 (dt, J = 4, 48 Hz, 2H), 4.70 (dt, J = 4, 28 Hz, 2H); 13C NMR (200 MHz, DMSO-d6) δ 167.38, 162.01, 142.81, 131.76, 125.66, 123.68, 114.93, 82.35 (d, J = 1.67 Hz), 61.61, 50.50 (d, J = 0.19 Hz); m.p. 179.5-180.5 ºC.

Synthesis of 6

A mixture of N-hydroxysulfosuccinimide sodium salt (0.47 g, 2.16 mmol), DCC (0.48 g, 2.33 mmol), and the benzoic acid derivative 2[28] (0.37 g, 2.10 mmol) in DMF (5 mL) was stirred for 24 h at room temperature. After white solids were removed by filtration, the reaction mixture was acidified by 0.5 N HCl (5 mL), diluted with water (80 mL), and then purified by solid phase extraction using a Waters Oasis® HLB cartridge (3 g). The collected product was purified again using the HLB cartridge, and the final product was obtained as a white solid (97 mg) after lyophilization. 1H NMR (400 MHz, DMSO-d6) δ 8.04 (d, J = 8 Hz, 2H), 7.18 (d, J = 8 Hz, 2H), 4.94 (d, J = 2 Hz, 2H), 4.00 (m, 1H), 3.65 (t, J = 2 Hz, 1H), 3.20 (br, 1H), 2.88 (m, 1H); 13C NMR (200 MHz, DMSO-d6) δ 169.41, 165.94, 163.06, 132.78, 116.12, 79.53, 78.77, 56.76, 56.42, 31.42; m.p. 150 °C, decomposition. Anal. Calcd for C16H15FN4O8S•0.5H2O: C, 42.58; H, 3.57; N, 12.41. Found: C, 42.72; H, 3.47; N, 12.52.

Synthesis of 7

General method of Cu(I)-catalyzed click reaction: A solution of 2-fluoroethyl azide (0.23 mmol) in DMF (0.5 mL) was added to a 10 mL screw-cap tube containing the sulfo-NHS alkyne

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derivative 6 (20 mg, 0.073 mmol), and followed by addition of Cu(I)/BPDS (100 µL from a mixture of 5 mg CuSO4•5H2O in 50 µL water, 15 mg sodium ascorbate in 50 µL water, and 6 mg BPDS 100 µL in 4:1 water/DMF). The reaction was complete within 5 min according to HPLC analysis. The reaction mixture was diluted with 0.2 % TFA (4.5 mL), and purified by reversed phase HPLC to afford final product as a white solid (15 mg) (HPLC column: Nucleosil, C18 250×16 7 µ; mobile phase: 17% MeCN/83% water/0.1% TFA; Flow rate: 8 mL/min; Retention time: 15 min). 1H NMR (400 MHz, DMSO-d6) δ 8.30 (s, 1H), 8.03 (d, J = 8 Hz, 2H), 7.25 (d, J = 8 Hz, 2H), 5.30 (s, 2H), 4.80 (dt, J = 4, 44 Hz, 2H), 4.70 (dt, J = 4, 28 Hz, 2H), 3.98 (m, 1H), 3.20 (br, 1H), 2.88 (m, 1H); 13C NMR (200 MHz, DMSO-d6) δ 169.45, 165.95, 142.46, 132.85, 125.82, 116.01, 82.33 (d, J = 1.67 Hz), 61.94, 56.78, 50.52 (d, J = 0.19 Hz), 31.44; mp 240 °C decomposition. Anal. Calcd for C14H11NO8S•3H2O: C, 41.28; H, 4.21; N, 3.44. Found: C, 41.47; H, 4.02; N, 3.60.

Synthesis of 9

A solution of 1 M Bu4NOH in water (0.52 mL) was azeotropically dried with MeCN (5 × 1 mL) under a flow of N2 at 105 °C. A suspension of 8[29] (115 mg, 0.52 mmol) in MeCN (5 mL) was added to the dried Bu4NOH to form a clear solution, into which a solution of N,N,N,N-tetramethyl-O-(N-succinimidyl)uronium tetrafluoroborate (TUSU) (160 mg, 0.53 mmol) in MeCN (1 mL) was added. The reaction was complete within 5 min according to HPLC analysis. The reaction mixture was purified by reversed phase HPLC to afford 9 as a white solid (105 mg). 1H NMR (400 MHz, DMSO-d6) δ 13.63 (br, 1H), 8.17 (s, 1H), 7.87 (s, 1H), 7.81 (s, 1H), 5.01 (d, J = 2 Hz, 2H), 3.66 (t, J = 2 Hz, 1H), 2.87 (s, 4H); 13C NMR (200 MHz, DMSO-d6) δ 169.03, 164.45, 159.79, 156.57, 132.34, 124.96, 121.89, 121.16, 118.60, 78.18, 77.14, 55.04,

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24.39; mp 172.0-174.0 ºC. Anal. Calcd for C15H11NO7: C, 56.79; H, 3.50; N, 4.42. Found: C, 56.63; H, 3.52; N, 4.54.

Synthesis of 10

The same method as for 7 was used except that as mobile phase 32% MeCN/68% water/0.1% TFA was used as for HPLC purification (TR = 14 min). Yield, 36%. 1H NMR (400 MHz, DMSO-d6) δ 13.55 (br, 1H), 8.26 (s, 1H), 8.15 (s, 1H), 7.91 (s, 1H), 7.87 (s, 1H), 5.34 (s, 2H), 4.76 (dt, J = 4, 44 Hz, 2H), 4.74 (dt, J = 4,28 Hz, 2H), 2.87 (s, 4H); 13C NMR (200 MHz, DMSO-d6) δ 170.62, 166.12, 161.45, 159.11, 142.65, 133.98, 126.63, 125.68, 123.17, 122.65, 120.13, 82.32 (d, J = 1.67 Hz), 62.32, 50.51 (d, J = 0.19 Hz), 26.00; m.p. 207.0-209.0 °C. Anal. Calcd for C17H15FN4O7: C, 50.25; H, 3.72; N, 13.79. Found: C, 49.88; H, 3.84; N, 13.43.

General procedure of Cu(I)-catalyzed click labeling using 2-[^{18}F]fluoroethyl azide.

To the 10 mL screw-cap Pyrex tube containing ~1 mg alkyne precursor was added 2-[^{18}F]fluoroethyl azide in DMF (250 µL) according to the literature, and followed by addition of Cu(I)/bathophenanthroline disulfonate (BPDS) (50 µL from a mixture of 5 mg CuSO4•5H2O in 50 µL water, 15 mg sodium ascorbate in 50 µL water, and 6 mg BPDS 100 µL in 4:1 water/DMF). The tube was shaken and then allowed to settle for 5-10 min to complete the reaction. 0.1% TFA or 10% MeCN/90% water/0.1% TFA (up to 4 mL) was added to the reaction mixture for HPLC purification using an Agilent SB-C18 column (5µm 250 × 9.4 mm) with a flow rate of 4 mL/min and UV at 240 nm and the specified mobile phase for each compound.

General procedure of conjugation and radiolabeling of PAMAM dendrimers

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Into a 500 μL solution of PAMAM dendrimers in a 1.5 mL microcentrifuge tube was added ^{18}F NHS ester in DMF (10 μL). The reaction mixture was immediately vortexed, and then allowed to react at room temperature. At specified time point, an aliquot of reaction mixture was analyzed by silica TLC.

Conclusion

In conclusion, two anionic NHS esters, **7** and **10**, were synthesized, and they were radiolabeled with ^{18}F using a Cu(I)-catalyzed click reaction with 2-[^{18}F]fluoroethyl azide. The treatment of primary amine-terminated PAMAM G6 dendrimers with [^{18}F]**7** and [^{18}F]**10** demonstrated highly efficient conjugation and radiolabeling reactions between the anionic NHS esters and the cationic PAMAM dendrimers, due to a favorable electrostatic interaction, the extent of which was greatly affected by solvent and medium effects. The strategy using electrostatic interactions to facilitate the labeling of macromolecules has great potential for improving the conjugation and labeling efficiency of both PAMAM dendrimers and other nanoparticles with a variety of interesting modifying groups.

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