Synthesis of Branched Monodisperse Oligoethylene Glycols and \(^{19}\text{F} \text{MRI-Traceable Biomaterials through Reductive Dimerization of Azides}

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1. General information

$^1$H, $^{19}$F and $^{13}$C NMR spectra were recorded on a Bruker 400 MHz. Chemical shifts are in ppm and coupling constants ($J$) are in Hertz (Hz). $^1$H NMR spectra were referenced to tetramethylsilane (d, 0.00 ppm) using CDCl$_3$ as solvent, $^{13}$C NMR spectra were referenced to solvent carbons (77.16 ppm for CDCl$_3$). $^{19}$F NMR spectra were referenced to 2% perfluorobenzene (s, -164.90 ppm) in CDCl$_3$. The splitting patterns for $^1$H NMR spectra are denoted as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), b (broad) and combinations thereof. MS spectra were recorded on an Asilent LCMS-1100 spectrometer. MALDI-TOF mass spectra were recorded on a MALDI-TOF/TOF 5800 (AB SCIEX) spectrometer using the reflector mode for positive ions with $\alpha$-cyano-4-hydroxycinnamic acid as matrix.

2. n-Octanol/Water partition coefficients (logP) measurement

The logP values of compounds 40-41 were measured following shake-flask method. Briefly, the product was dissolved in distilled $n$-octanol saturated with water. Then 1 mL of this solution was mixed with an equal volume of water saturated with distilled $n$-octanol and mixed on a vortex device. After shaking the mixture overnight, water phase was separated by centrifugation. Equal-volume samples of the shaken water phase and the starting solution were subsequently taken and analyzed by HPLC. The peak area was measured at $\lambda = 254$ nm, and compared with calibration curve to obtain the concentration of the peptide. LogP values were determined from: $\text{Lg}\left[\frac{C_s-C_w}{C_w}\right]$, where $C_s$ and $C_w$ are the concentrations of the starting water solution and the water phase of the compound, respectively.

3. Solvent-dependent $^{19}$F NMR of 40 and 41

Solvent-dependent $^{19}$F NMR spectra were referenced to 10% sodium trifluomethanesulfonate (s, -79.61 ppm) in D$_2$O at 25°C.
4. Dynamic light scattering of 41

Solution of 41 in H2O at 0.07 mM was used for DLS analysis. The particle size was measured at an angle of 90° in a 10 mm diameter cell at the room temperature with a Dynamic Light Scattering (DLS) Analyzer (Malvern ZetasizerNano 3690). Eleven scans were run for each measurement and the measurement was repeated 3 times. The particle size and polydispersity index (PDI) were calculated by Malvern software.

5. In vitro 19F MRI experiments of 40 and 41

All magnetic resonance imaging (MRI) experiments were performed on a 400 MHz Bruker BioSpec MRI system. The temperature of the magnet room was maintained at 25 °C during the entire MRI experiment. The 19F in vitro images were acquired using a gradient-echo (GRE) pulse sequence, method = RARE, matrix size = 32 × 32, SI = 20 mm, FOV = 3.0 cm, TR = 2500 ms, TE = 2.8 ms, scan time = 160 s.

6. Cytotoxicity assay of 40 and 41

HepG2 cells were cultured in DMEM medium containing 10% FBS and 1% streptomycin double antibody. L929 cells were cultured in alpha-MEM medium containing 10% FBS and 1% streptomycin double antibody. All cells were cultured at 37 °C in humidified atmosphere containing 5% CO2 and the growth medium was replaced with fresh media every 24 h. The cell viability assay of compounds 40 and 41 were investigated in L929 cell lines and HepG2 cell lines in vitro by MTT assay. L929 cells HepG2 cells were seeded into a 96-well plate for several hours. Subsequently, a gradient concentration of the compounds ranging from 62 μg/mL to 1000 μg/mL were added in a series of wells. Every concentration was set with five wells at least. The wells with 100 μL culture medium alone were used as negative control and wells containing cells alone were used as positive control. After incubation for 24 h, the medium was replaced with 100 μL MTT (1.0 mg/mL) solution and incubated for 4 h. Then the medium was replaced with 200 μL DMSO and the absorbance value was measured at 490 nm using a microplate reader (Bio Tek Instruments, USA).
7. Copies of $^1$H/$^13$C/$^{19}$F NMR, MS and mass spectra (HRMS) of compounds

$^1$H NMR of compound 2

$^1$H NMR of compound 3
$^1$H NMR of compound 4

$^1$H NMR of compound 5
$^1$H NMR of compound 6

$^{13}$C NMR of compound 6
HRMS of compound 6

\[
\begin{align*}
\text{ESI Scan (0.083 min)} & \quad \text{Frag=70.0V 8PEG-20200417+. d Subtract} \\
611.18844 & \\
242.28565 & \\
751.26941 & \\
1197.35859 & 
\end{align*}
\]

\[\text{Counts vs. Mass-to-Charge (m/z)}\]

\(0 \quad 100 \quad 200 \quad 300 \quad 400 \quad 500 \quad 600 \quad 700 \quad 800 \quad 900 \quad 1000 \quad 1100 \quad 1200 \quad 1300 \quad 1400\]

\[\text{x10}^4\]

\(^1\text{H} \text{NMR of compound 7}\)

\[\text{N}\text{3CH2CHO} \text{Me}\]

\(^1\text{H} \text{NMR} (\text{CDCl}_3, 400 \text{ MHz})\]
$^1$H NMR of compound 8

$^1$H NMR of compound 9
$^1$H NMR of compound 10

$^{19}$F NMR of compound 10
$^1$H NMR of compound 11

$^{19}$F NMR of compound 11
$^1$H NMR of compound 12

$^{13}$C NMR of compound 12
HRMS of compound 12

\[ \text{RT: 6.55 AV: 1 NL: 1.02E7} \]

\[ \text{T: FTMS + p ESI Full ms [150.0000-2000.0000]} \]

\( m/z \) values:
- 744.4346
- 745.4379
- 750.4084
- 746.4404
- 752.4630
- 758.4476
- 751.4637
- 728.9197
- 731.5043
- 752.4939
- 756.4050
- 752.4630
- 768.4216
- 772.8856
- 741.9021
- 739.9115

\[ \text{1H NMR of compound 13} \]

\[ \text{1H NMR (CDCl₃, 600 MHz)} \]
$^{13}$C NMR of compound 13

HRMS of compound 13
$^1$H NMR of compound 14

$^{13}$C NMR of compound 14
HRMS of compound 14

1H NMR of compound 15
$^{13}$C NMR of compound 15

HRMS of compound 15
$^1$H NMR of compound 16

$^{13}$C NMR of compound 16
HRMS of compound 16

1H NMR of compound 17
$^{13}$C NMR of compound 17

HRMS of compound 17
$^1$H NMR of compound 18

$^{13}$C NMR of compound 18
$^{19}\text{F NMR of compound 18}$

$^{19}\text{F NMR (CDCl}_3, 370\text{MHz})$

$^{19}\text{F NMR of compound 18}$

$^{19}\text{F NMR (CDCl}_3, 370\text{MHz})$
\[ \text{\textsuperscript{1}H NMR of compound 19} \]

\[ \text{\textsuperscript{13}C NMR of compound 19} \]
$^{19}$F NMR of compound 19

HRMS of compound 19
$^1$H NMR of compound 20

$^{13}$C NMR of compound 20
HRMS of compound 20

\[ \text{RT: 7.08, AV: 1, NL: 1.31E9} \]
\[ \text{T: FTMS + p ESI Full ms [150.0000-2000.0000]} \]

\[ \text{m/z} \]

\[ \text{Relative Abundance} \]

\[ \text{524.2828, 526.2884, 502.3006, 531.8671, 519.3280, 540.2559, 564.2752, 504.3057, 592.2700, 578.2905, 462.9408, 547.3603, 469.0003} \]

\[ \text{^1H NMR of compound 21} \]

\[ \text{^1H NMR (CDCl3, 400 MHz)} \]
$^{13}$C NMR of compound 21

HRMS of compound 21
$^1$H NMR of compound 22

$^{13}$C NMR of compound 22
HRMS of compound 22

$\text{M}^+$

$\text{RT: 7.61}
\text{AV: 1}
\text{NL: 2.93E8}
\text{T: FTMS + p ESI Full ms [150.0000-2000.0000]}$

$\text{m/z: 876.4919, 877.4952, 878.4980, 871.5369, 879.5003, 892.4659, 880.5038, 832.4661, 854.5120, 888.2886, 861.4458}$

$\text{1H NMR of compound 23}$

$\text{H NMR (CDCl$_3$, 400 MHz)}$

$\text{H}$
$^{13}$C NMR of compound 23

HRMS of compound 23
$^1$H NMR of compound 24

$^{13}$C NMR of compound 24
HRMS of compound 24

1H NMR of compound 25
$^{13}$C NMR of compound 25

HRMS of compound 25
$^1$H NMR of compound 26

$^{13}$C NMR of compound 26
HRMS of compound 26

$^{1}$H NMR of compound 27
$^{13}$C NMR of compound 27

HRMS of compound 27
$^1$H NMR of compound 28

$^{13}$C NMR of compound 28
HRMS of compound 28

1H NMR of compound 29
$^{13}$C NMR of compound 29

HRMS of compound 29
$^1$H NMR of compound 30

$^{13}$C NMR of compound 30
HRMS of compound 30

\[
\begin{array}{l}
\text{m/z} \quad \text{Relative Abundance} \\
730.3981 \quad 100 \\
732.4040 \quad 75 \\
746.3717 \quad 65 \\
760.4088 \quad 55 \\
725.4432 \quad 45 \\
686.3721 \quad 35 \\
748.3738 \quad 25 \\
702.9202 \quad 15 \\
758.9288 \quad 5 \\
734.4089 \quad 1 \\
762.4174 \quad 0.5 \\
672.9072 \quad 0.5 \\
772.4669 \quad 0.5 \\
678.9644 \quad 0.5 \\
716.9432 \quad 0.5 \\
800.9197 \quad 0.5 \\
708.9736 \quad 0.5 \\
694.9557 \quad 0.5 \\
790.9132 \quad 0.5 \\
778.9296 \quad 0.5 \\
\end{array}
\]

1H NMR of compound 31

[Image of NMR spectrum]
$^{13}$C NMR of compound 31

HRMS of compound 31
\(^1\)H NMR of compound 32

\(^{13}\)C NMR of compound 32
HRMS of compound 32

H NMR of compound 33
$^{13}$C NMR of compound 33

HRMS of compound 33
$^1$H NMR of compound 34

$^{13}$C NMR of compound 34
$^{19}$F NMR of compound 34

HRMS of compound 34
$^1$H NMR of compound 35

$^{13}$C NMR of compound 35
$^{19}$F NMR of compound 35

HRMS of compound 35
$^1$H NMR of compound 36

$^{13}$C NMR of compound 36
$^{19}$F NMR of compound 36

HRMS of compound 36
\(^1\)H NMR of compound 37

\(^{13}\)C NMR of compound 37
$^{19}\text{F NMR of compound 37}$

$^{19}\text{F NMR (CDCl}_3, 270\text{MHz)}$

$\text{HRMS of compound 37}$

$\text{RT: 10.62 AV: 1 NL: 2.73E7}$

$T: \text{FTMS + p ESI Full ms [150.0000-2000.0000]}$
$^1$H NMR of compound 38

$^{13}$C NMR of compound 38
$^{19}$F NMR of compound 38

HRMS of compound 38
$^1$H NMR of compound 39

$^{13}$C NMR of compound 39
$^{19}$F NMR of compound 39

HRMS of compound 39
$^1$H NMR of compound 40

$^{13}$C NMR of compound 40
$^{19}$F NMR of compound 40

HRMS of compound 40
$^1$H NMR of compound 41

$^{13}$C NMR of compound 41
$^{19}$F NMR of compound 41

HRMS of compound 41