

N_3S

A New Synthetic Method of N_3S -type Chelators for Radioisotope Labeling

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215-4

Tc-99m	MAG3	N_3S	Chelator
			MAG2-NHS
MAG3, MAG2Gaba, MAG2Gaba-Biocytn, MAG2-Biocytn			
Tc-99m	Re-188		chelator

Abstract

A simple route for the facile synthesis of MAG3-type N_3S bifunctional ligands which are very widely used in nuclear medicine field was developed. By this simple method MAG3, MAG2GABA, MAG2GABA-Biocytn, MAG2-Biocytn was obtained with 70-80% yield from key intermediate MAG2-NHS. And MAG2Gaba was radiolabelled with ^{99m}Tc and ^{188}Re and were compared with MAG3.

I.

DNA	^{99m}Tc	^{188}Re
		target
.1) 2) 3).		
	in-vivo	in-vitro
		가
	chelation	

MAG3 type chelator

N- S- 가

가 , C-
(active ester)

Tc-99m

Mercaptoacetyltriglycine(MAG3)

.4) Fritzberger

S-protected

thioglycolic acid active ester triglycine

coupling 5)

가 gly-gly-gaba

가

N-

S-protected thioglycolic acid

N-terminal protecting group

Succinimidyl S-protected mercapto-

acetylglcylglycinate

MAG3

MAG2GABA

amidothiol ligand

2. MAG2-NHS

chelator

Scheme I

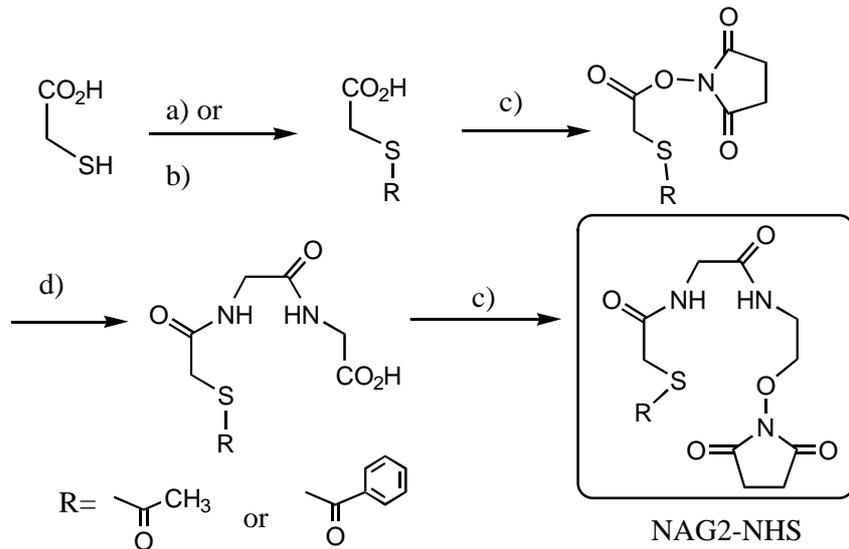
chelator

MAG2-NHS

modify

scheme (II)

Scheme II. Key Intermediate



a)BzCl, NaOH, 10

b) AcCl, rt

c) N-Hydroxysuccinimide, DCC

d)diglycine, NaHCO3

S-Protected mercaptoacetic acid

N-hydroxy succinimidyl ester

glycylglycine

coupling

N-hydroxysuccinimide

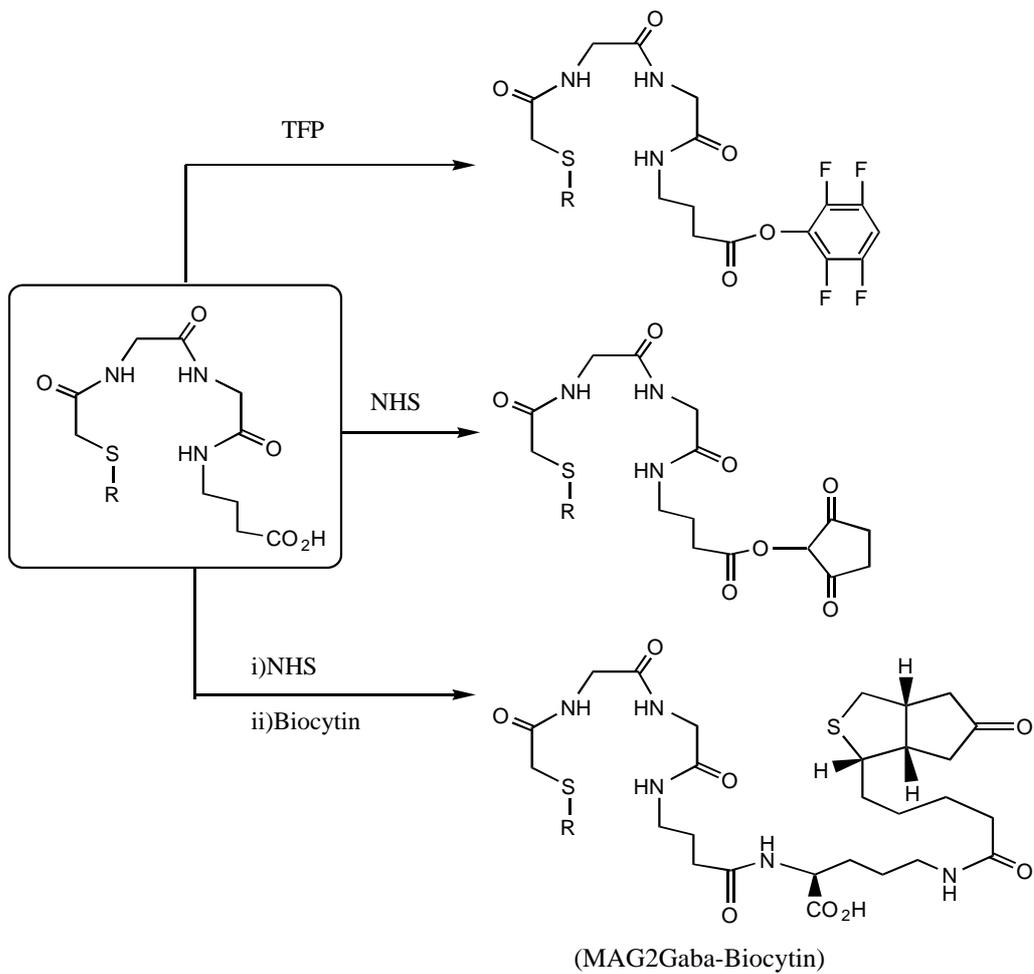
DCC

isopropanol

benzoyl acetyl Sulfur protecting group
 가 , S-Benzoyl MAG2-NHS S-Acetyl MAG2-NHS
 S-Acetyl protected chelator S-Benzoyl protected chelator pH
 가 protein antibody biomolecule
 -aminobutyric acid coupling
 MAG2GABA MAG3 24-30 % (5step from thioglycolic acid, unoptimized)
 1H NMR electron spray ionization MASS

3. C- functionalization
 MAG2GABA C- functionalization
 , tetrafluorophenyl ester NHS ester pretargeting method
 MAG2GABA-Biotin 77.4%(2step overall from MAG2GABA)
 (Scheme III).

Scheme III. Functionalization of C-terminal



pH 1 . white precipitate ice-water bath 30
 filter , crude 94.64g . crude
 product Ethyl acetate 50ml 51.15g(52.1%) .
 m.p =106-108 1H NMR (CDC13) 7.47-8.0 (m, 5H) 3.92 (s, 2H)

Succinimidyl S-benzoylthioglycolate

S-Benzoylglycolic acid(51.15g, 0.26moles) N-hydroxysuccinimide (31.41g, 1.05eq)
 dimethoxyethane 300ml ice-salt bath -5-0
 30 dicyclohexylcarbodiimide(56.32g, 1.05eq) DME 가 .
 -5-0 2 , 16 filter
 dicyclohexylurea filtrate evaporation ethyl
 acetate 150ml 40.364g (52.9%) .
 m.p =135-141 1H-NMR (DMSO-d6) 8.0-7.5 (m, 5H) 4.45 (s, 2H) 2.8 (s, 4H)

N-(S-Benzoylmercaptoacetyl)-glycylglycine (4)

Succinimidyl-S-Benzoylthioglycolate(3)(23.3g, 0.0794moles) anhydrous acetone 250ml
 (가 , 50) 500ml one-neck flask glycylglycine
 10.49g (0.0794moles, 2eq.) Sodium bicarbonate 13.3g(0.1588moles) 100ml
 . glycylglycine succinimidyl active ester acetone
 가 . 1.5 TLC check , Acetone evaporation
 aqueous c-HCL pH 3.0 overnight standing
 . filter solid 2 EtOH 1
 Vacuum Pump drying Acetonitrile 160ml 5 reflux
 filter 30.391g(89.0%) .
 m.p =190-191 1H-NMR (DMSO-d6) 7.42-7.99 (m, 5H) 8.23 (t, 1H) 8.54 (t, 1H) 3.90 (s, 2H)
 3.78 (d, J=5.8Hz, 4H)

Succinimidyl-N-(S-benzoylmercaptoacetyl)glycylglycinate

1L 2-neck round-bottomed flask N-(S-benzoyl mercaptoacetyl)glycylglycine
 (18.61g, 0.06moles) N-ydroxysuccinimide (7.12g, 0.06moles) DMF DME. 1:1
 200ml . ice-water bath . And this
 solution was cooled in a ice bath. To this chilled mixture was added Dicyclohexylcarbodiimide (12.5g,
 0.06moles) dimethoxyethane 30ml 30 dropping funnel 가 .
 0 2 16 dicyclohexylurea
 50ml . ethyl acetate

50ml crystallization 13.07g first crop filtrate
 isopropanol second crop 8.3g first crop second crop
 isopropanol 19.1g(78.2%)
 m.p = 144-148 1H-NMR(DMSO-d6) 8.60 (brt, 2H) 4.30 (d, J=5.12Hz, 2H) 3.91 (s, 2H) 3.82 (d, J=5.22Hz, 2H) 2.83 (s, 4H)

N-(S-benzoylmercaptoacetyl)-glycylglycyl-GABA(MAG2GABA)

-aminobutyric acid (1.57g, 0.015moles, 1.01eq.) Sodium bicarbonate (2.53g, 0.03moles, 2.0eq.)
 30ml flask MAG2-NHS(6.11g, 0.015moles) dimethoxyethane 30ml
 DMF 7ml CO2 가
 가 . 2 Dimethoxyethane
 c-HCl 가 pH 2.0 precipitate
 filter crude product . acetonitrile 4.61g
 (77.7%)

TLC (R.f=0.21, CHCl3:MeOH=4:1, UV-active, PMA-black,)

m.p = 158-165 1H-NMR (DMSO-d6) 12.5 (br, 1H) 8.51 (t, J=5.5Hz, 1H) 8.13 (t, J=5.8Hz, 1H)
 7.94-7.54 (m, 5H) 7.74 (t, J=5.5Hz, 1H) 3.89 (s, 2H) 3.76 (d, J=5.59Hz, 2H) 3.67 (d, J=5.83Hz, 2H) 3.06
 (q, J=6.5Hz, 2H) 2.20 (t, J=7.46Hz, 2H) 1.62 (q, J=7.20Hz, 2H)

Tetrafluorophenyl N-(S-benzoylmercaptoacetyl)glycylglycyl- -amino butyrate (MAG2GABA-THP)

N-(S-benzoylmercaptoacetyl)-glycylglycyl- -aminobutyric acid (1g, 2.5mmoles) tetrafluoro
 phenol (0.462g, 0.0028moles, 1.1eq.) DMF 25ml , dicyclohexylcarbodiimide (0.57g,
 2.75mmoles) 가 . 18 filter
 filtrate ethyl acetate (60ml) 381mg (28.1%
)

m.p=174-180 1H-NMR(DMSO-d6) 8.53 (t, 1H) 8.16 (t, 1H) 7.94-7.54 (m, 5H) 7.93 (m, 1H)
 7.80 (t, J=5.66Hz, 1H) 3.89 (s, 2H) 3.76 (d, J=5.60Hz, 2H) 3.69 (d, J=5.86Hz, 2H) 3.16 (q, J=5.90Hz,
 2H) 2.78 (t, J=7.46Hz, 2H) 1.80 (q, J=7.18Hz, 2H)

Succinimidyl Bz-MAG2- -aminobutyrate(MAG2GABA-NHS)

2.965g(7.5mmoles) Bz-MAG2GABA 0.89g(7.5mmoles) of N-hydroxy succinimide
 dimethylformamide 35ml dimethoxyethane 7ml dicyclohexylcarbodiimide (1.563g,
 7.5mmoles) dimethoxyethane(5ml) 가 15 .
 dicyclohexylurea filter isopropanol 20ml

5 reflux 가 filter chromatographically pure

3.24g (87.8%)

R.f = 0.69 (CHCl₃ : MeOH = 4 : 1 , UV) m.p.=174-177 (with decompose) 1H-NMR (DMSO-d₆)

8.60 (t, 1H) 8.20 (t, 1H) 7.95-7.55 (m, 5H) 7.87 (t, J=5.66Hz, 1H) 3.91 (s, 2H) 3.78 (d, J=5.62Hz, 2H) 3.70 (d, J=5.80Hz, 2H) 3.15 (q, J=6.5Hz, 2H) 2.82 (s, 2H) 2.69 (t, J=7.60, 2H) 1.77 (q, J=7.2Hz, 2H)

Bz-MAG2GABA-Biocytin

Biocytin(372.5mg, 1mmoles) sodium bicarbonate(168mg, 2mmoles) 5ml

Bz-MAG2GABA succinimidyl ester (492.44mg, 1mmoles) dimethyl formamide 7ml

가 . 15 pH 2.0

precipitate acetonitrile

660mg (88%)

m.p= 194-196(decomp.) 1H-NMR (DMSO-d₆) 8.49 (t, 1H) 8.12 (t, 1H) 8.0 (d, J=7.73Hz, 1H) 7.95-7.56 (m, 5H) 7.75 (t, 1H) 6.38, 6.33 (s, 2H) 4.29 (m, 1H) 4.13 (m, 1H)

3.89 (s, 2H) 3.77 (d, J=5.63Hz, 2H) 3.67 (d, J=5.86Hz, 2H) 3.10 (m, 1H) 3.05 (q, J=6.7Hz, 2H) 3.00 (q, J=6.6Hz) 2.83 (dd, J=12.4, 5.11Hz, 1H) 2.57 (d, J=12.4Hz, 1H) 2.12 (t, J=7.50Hz, 2H) 2.04 (t, J=7.4Hz) 1.2-1.6 (m, 14H)

MASS (ESI) m/z 750.57(M++1)

N-(S-Benzoylmercaptoacetyl)-triglycine

m.p= 192-196 (decomp.) 1H-NMR (DMSO-d₆) 12.5 (br, 1H) 8.51 (t, 1H) 8.15-8.25 (2t, 2H) 8.0-7.5 (m, 5H) 3.9 (s, 2H) 3.9-3.7 (m, 6H)

Succinimidyl N-(S-Benzoylmercaptoacetyl)-triglycinate (MAG3-NHS ester)

Succinimidyl-MAG2GABA isopropanol 69.0%

m.p=146-149 1H-NMR (DMSO-d₆) 8.5-8.6 (2t, 2H) 8.25-8.35 (t, 1H) 8.0-7.5 (m, 5H) 4.3 (d, 2H) 3.9 (s, 2H) 3.8 (m, 4H) 2.8 (s, 4H)

N-(S-Acetylmercaptoacetyl)-glycylglycyl- -aminobutyrate(MAG2GABA)

S-Benzoyl-MAG2GABA

m.p=185-190(with decompose) 1H-NMR (DMSO-d₆) 8.45 (t, 1H) 8.25 (t, 1H) 7.7(t, 1H) 3.76 (d, 2H) 3.7 (s, 2H) 3.6 (d, 2H) 3.05 (q, 2H) 2.3(s,3H) 2.20 (t, 2H) 1.62 (quint, 2H)

III.

MAG3-type chelator
biocytin coupling MAG2-Biocytin avidin-biotin high binding
affinity radioimmuno therapy
MAG3 가
, N₃S chelator C-
residue 가

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