SUPPORTING INFORMATION

A novel radiolabelled salmochelin derivative for bacteria-specific PET imaging: synthesis, radiolabeling and evaluation

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SN and AF planned the project and are responsible for funding acquisition. RM performed chemical synthesis and radiolabelling and together with AF analysed chemical data. SN, SS, and SH planned, performed, and analysed *in vitro* and *in vivo* experiments. SN and AF wrote the manuscript with input from all authors. We thank B. Schuhen, M. Brück, S. Bouma, S. Höppner, S. Köster, A. Niehues, D. Reinhardt, R. Priebe, C. Bätza, C. Essmann for excellent technical assistance.

Content

1. General information and materials

Unless otherwise stated, all solvents and reagents were used as received from commercial suppliers without further purification. Reaction progress was monitored by TLC performed on aluminum plates coated with silica gel 60 F_{254} from Merck. Chromatograms were visualized by fluorescence quenching with UV light at λ=254 nm and by staining with iodine vapour or staining solutions. TLC staining solutions were prepared as follows: Stain A: 2 mL p-anisaldehyde and 8 mL conc. H_2SO_4 were dissolved in 200 mL EtOH abs.; Stain B: 8 g $Ce(SO_4)_2$*$2H_2O$ was dissolved in 100 mL 15% aq. sol. H_2SO_4; Stain C: 0.6 g ninhydrin was dissolved in 200 mL abs. EtOH and 6 mL conc. AcOH was added.

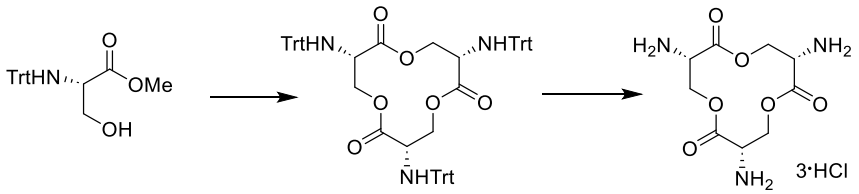
SPE, column filtrations and column chromatography were performed manually on self-made cartridges or columns with ultrapure 60Å silica gel 60-200 µm from Acros or neutral alumina (EcoChrom NP Alumina N) with reduced activity to II/III by adding 4.5% water. During the trituration process ultrasound bath Bandelin Sonorex RK 100H was used. For separation of solids, Eppendorf Centrifuge 5804R was used with cooling set to 6 °C. Melting point determination was performed using a Mettler Toledo MP70 melting point system in open capillary tubes and the given values are uncorrected.

All mass and NMR spectra were obtained from the Mass Spectrometry and NMR Departments of the Organic Chemistry Institute at the University of Münster. Mass spectra were obtained using a Bruker MicrOTof or Autoflex Speed MALDI-TOF, Thermo Fisher Scientific Orbitrap LTQ XL or Orbitrap Velos Pro spectrometers. ^{1}H-NMR and ^{13}C-NMR spectra were recorded using Bruker Neo 400, Agilent DD2 500 and DD2 600 spectrometers at 300K. Chemical shifts are reported in parts per million (ppm, δ), relative to TMS (δ = 0.00 ppm). If TMS is not present, shifts are referenced to the solvent peak of $CDCl_3$ (^{1}H: δ = 7.26 ppm; ^{13}C: δ = 77.16 ppm), MeOH-d_4 (^{1}H: δ = 3.31 ppm; ^{13}C: δ = 49.00 ppm) or DMSO-d_6 (^{1}H: δ = 2.50 ppm; ^{13}C: δ = 39.52 ppm). Coupling constants are given in Hz (J). ^{1}H and ^{13}C NMR splitting patterns are signed as singlet (s), doublet (d), triplet (t), quartet (q) as they appeared in the spectrum. Splitting patterns that could not be interpreted or visualized easily are signed as multiplet (m) or broad (br).

Radioactivity measurements were performed on Isomed 2010-activimeter from MED Nuklearmedizintechnik, Dülmen, Germany. Total radioactivity for the determination of experimental log*D*-value was measured using a gamma-counter Wizard^2 2480 from PerkinElmer. Centrifugation of samples for all *in vitro* characterisation measurements was done in MCF-2360 centrifuge from LMS Consult GmbH. Samples for the serum stability determination were incubated using a thermo shaker PST-60 HL plus from Kisker Biotech GmbH. Radiochemical purity was determined by scanning developed TLC plates on thin layer radio-chromatograph (TLC-scanner) miniGita Elysia-Raytest as well as analysing HPLC chromatograms. They were made on Knauer HPLC system equipped with two Smartline 1000 pumps, UV detector Smartline 2500, gamma-detector Raytest Isotopenmessgeräte and column Waters XBridge Amide, 150 x 4.6 mm, 3.5µm with the following HPLC method: Mobile phase A = ACN; mobile phase B = H_2O; Flow = 1,5 mL / min; UV detector λ = 220 nm; γ-Counter units = cpm; Gradient 0 min – 0 % B, 2 min – 0 % B, 6 min – 90 % B, 8 min – 90 % B, 10 min – 0 % B, 12 min – 0 % B.

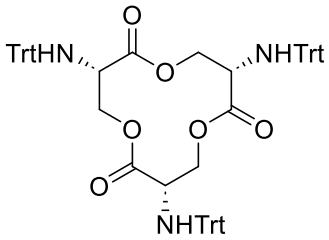
2. Total synthesis of Salmochelin

2.1 Preparation of serine trilactone precursor (3)



Scheme S1: Synthesis of the three membered macrolactone 3. Reagents and conditions: a) 2,2-Dibutyl-[1,3,2]-dioxastannolane, xylene, reflux, 24h; b) 4M HCl(g)/dioxane, DCM, rt, overnight.

Tris(*N*-trityl-L-serine)trilactone (2)



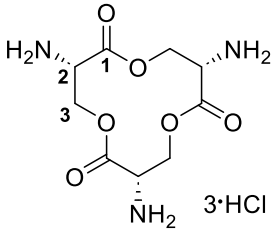
N-Trityl-L-serine methyl ester **1** (20.0 g, 55.4 mmol, 1.0 eq.) and 2,2-dibutyl[1,3,2]dioxa-stannolane (1.62 g, 5.54 mmol, 0.1 eq.) were dissolved in xylene (300 mL). Reaction flask was connected with Soxhlet extractor containing dry 4Å molecular sieves. Reaction mixture was refluxed for 24 hours and after that evaporated. Residual yellow solid was twice triturated with ether and dried on vacuum to give 15.2 g of product **2** (15.4 mmol, 84%) as a pale yellow solid.

^{1}H and **^{13}C{^{1}H} NMR**: match data from lit.[1]

TLC (silica, DCM, det.: UV_{254nm} & Stain B): $R_f = 0.88$

HRMS (ESI+): exact mass calculated for $[M+Na]^+$ ($C_{66}H_{57}N_3O_6Na$) required *m/z* 1010.41396; found *m/z* 1010.41462.

L-serine trilactone trihydrochloride (3)



Compound **2** (10.2 g, 10.3 mmol, 1.0 eq.) was dissolved in dry DCM (dried overnight over molecular sieves 4Å). At room temperature 4M $HCl_{(g)}$ in dioxane (23 mL, 92.7 mmol, 9.0 eq.) was added dropwise and reaction mixture was stirred on room temperature overnight. The reaction mixture was evaporated (at 30°C), residual solid was triturated twice with pentane and twice with diethyl ether and dried on vacuum to give 3.7 g of product **3** (10.1 mmol, 98%) as a very hygroscopic pale yellow solid.

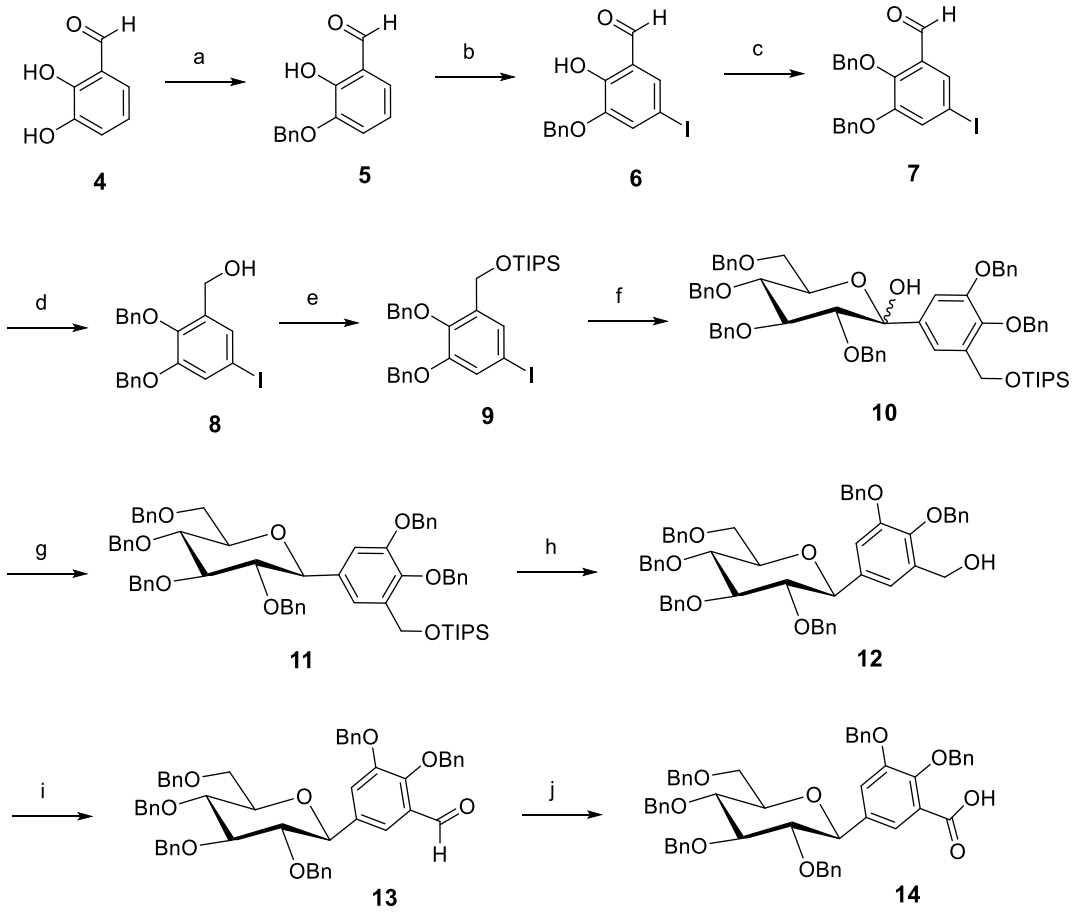
^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 9.39 (s, 9H, 3 NH_3^+) 5.12 (dd, 3H, *J*=12.5, 2.0 Hz, 3-H_β), 4.60 (bs, 3H, 3-H_α), 4.34 (dd, 3H, *J*=12.5, 2.7 Hz, 2-*H*)

^{13}C{^{1}H} NMR (75 MHz, DMSO-d_6): δ (ppm) = 165.4 (C-1), 63.2 (C-2), 53.0 (C-3).

HRMS (ESI+): exact mass calculated for $[M+H]^+$ ($C_9H_{16}N_3O_6$) required *m/z* 262.1034; found *m/z* 262.1036.

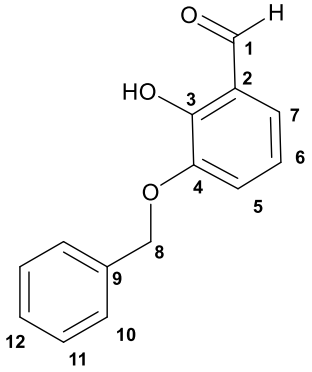
[1] R.J.A. Ramirez, L. Karamanukyan, S. Ortiz and C.G. Gutierrez, *THL*, 1997, **38**, 749.

2.2 Preparation of perbenzylated C-glucosyl-aryl carboxylic acid (14)



Scheme S2: Perbenzylated C-glucosyl-aryl carboxylic acid. Reagents and conditions: (a) BnBr (1eq.), NaH, THF, rt ; (b) ICl, $AgNO_3$, Py, $CHCl_3$; (c) BnBr, K_2CO_3, Me_2CO, reflux, 2h ; (d) $NaBH_4$, MeOH, 0°C->rt, 3h ; (e) TIPSCl, imidazole, DCM, rt ; (f) n-BuLi, PhMe, THF, -78°C ; tetra-O-Bn-D-glucono-1,5-lactone ; $AcOH_{(glac.)}$; $NaHCO_{3(aq)}$, -78°C->rt ; (g) Et_3SiH, BF_3*OEt_2, DCM, -78°C ; (h) conc. $HCl_{(aq)}$, EtOH, 50°C ; (i) DMP, DCM, rt ; (j) $NaClO_2$, NH_3SO_3, dioxane, H_2O, rt

a) 3-(Benzyloxy)-2-hydroxybenzaldehyde (5)



Sodium hydride 60% dispersion in mineral oil (5.4 g, 135.8 mmol, 2.5 eq.) was suspended in anhydr. THF (55 mL) and at room temperature dropwise was added solution of **4** (7.5 g, 54.3 mmol, 1.0 eq.) in anhydr. THF (25 mL). Reaction mixture was stirred[2] at room temperature for one hour and then benzyl bromide (6.5 mL, 54.3 mmol, 1.0 eq.) diluted with anhydr. THF (10 mL) was added dropwise. Stirring was continued at room temperature overnight. After completion of the reaction[3], reaction mixture was poured on ice. Obtained dark brown mixture was washed with pentane

[2] Very viscous suspension was formed. For efficient stirring it´s recommended to use larger volume of reaction flask and larger magnetic stirrer or mechanical stirrer.

[3] tracking by TLC (silica, EtOAc / cyclohex. 1:4 v/v, det.: UV_{254nm} & Stain B)

(100 mL) and ether (100 mL). Aqueous layer was acidified to pH = 1-2 with 5% $HCl_{(aq)}$ and then extracted with EtOAc (3x100 mL). Combined EtOAc extracts were washed with brine and dried over anhyd. $MgSO_4$. The solvent was removed under reduced pressure to give 10.7 g of crude product which was then dissolved in mixture EtOAc / cyclohex. 1:4 v/v (200 mL). Into that solution was added silica (30-50 g), obtained suspension was gently stirred and then filtered through sinter. The filtrate was evaporated, residue was triturated with pentane and dried to give 5.43 g of product **5** (23.8 mmol, 44%) as pale yellow solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) = 11.02 (s, 1H, O*H*), 9.84 (s, 1H, C*H*O), 7.38-7.25 (m, 5H, 9-*H* – 12-*H*), 7.11 (dd, 1H, J=1.5Hz, 7.9Hz, 7-*H*), 7.05 (d, 1H, J=7.9Hz, 5-*H*), 6.82 (t, 1H, J=7.9Hz, 6-*H*), 5.12 (s, 2H, 8-*H*).

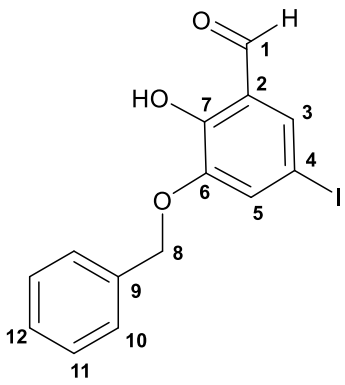
^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): δ (ppm) = 196.7 (C-1), 152.4 (C-4), 147.4 (C-3), 136.7 (C-9), 128.9, 128.3, 127.6, 125.5 (Ph-*C*H*)*, 121.3 (C-2), 121.2, 119.7 (Ph-*C*H), 71.6 (C-8).

NMR matches literature[4]

TLC (silica, EtOAc / cyclohex. 1:4 v/v, det.: UV_{254nm} & Stain A): R_f = 0.42

HRMS (ESI+): exact mass calculated for $[M+Na]^+$ ($C_{14}H_{12}O_3Na$) required *m/z* 251.06787; found *m/z* 251.06752.

b) 3-(Benzyloxy)-5-iodo-2-hydroxybenzaldehyde (6)[5]



Silver nitrate (343 mg, 2.0 mmol, 1.1 eq.) was dissolved in mixture $CHCl_3$/pyridine 2:1 v/v (4.5 mL) and at room temperature iodine monochloride (328 mg, 2.0 mmol, 1.1 eq.) dissolved in $CHCl_3$ (1 mL) was added dropwise. After stirring for 10 minutes at room temperature, **5** (418 mg, 1.8 mmol, 1.0 eq.) dissolved in $CHCl_3$ (1 mL) was added dropwise and stirring was continued next 2 hours.[6] Reaction mixture was diluted with $CHCl_3$/Et_2O 1:1 v/v (100 mL), stirred, filtered and filtrate was evaporated. Residue was dissolved in EtOAc (100 mL) and washed with 5% HCl_{aq} (30 mL), sat. sol. $Na_2S_2O_3$ (30 mL) and brine. Organic layer was dried over anhyd. $MgSO_4$. The solvent was removed under reduced pressure to give 606 mg of product **6** (1.7 mmol, 93%) as a yellow solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) = 10.95 (s, 1H, O*H*), 9.81 (s, 1H, C*H*O), 7.48-7.30 (m, 7H, Ph*H*), 5.12 (s, 2H, CH_2); match literature[5]

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[4] I.E. Wrona, A.E. Gabarda, G. Evano, and J.S. Panek, *J. of the Am. Chem. Soc.,* 2005, **127**, 15026-15027; K.A. Parker and A.T. Georges, *Org. Lett*., 2000, **2**, 497-499.

[5] Q. Zhang, C. Deng, L. Fang, W. Xu, Q. Zhao, J. Zhang, Y. Wang and X. Lei, *Chin. J. Chem.*, 2013, **31**, 355, modified method; A.V. Joshua, S.K. Sharma and D.N. Abrams, *Synth. Comm.*, 2008, **38**, 434, method only.

[6] tracking by TLC (silica, EtOAc / cyclohex. 1:4 v/v, det.: UV_{254nm} & Stain A); caution, longer reaction time makes lower yield.

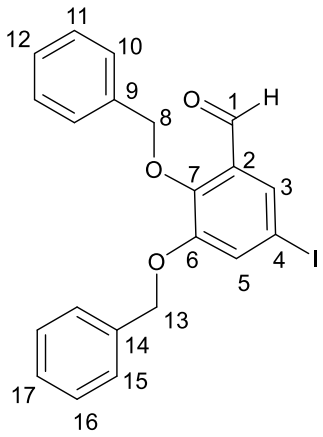
$^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ (ppm) = 195.3 (C-1), 152.4 (C-6), 148.4 (C-7), 135.9, 133.5 (both q-Ph*C)*, 128.9, 128.6, 127.8, 122.7 (all Ph*C*H), 79.8 (C-4), 71.8 (C-8).

TLC (silica, EtOAc / cyclohex. 1:4 v/v, det.: UV_{254nm} & Stain A): R_f = 0.51

mp 93 °C

HRMS (ESI+): exact mass calculated for $[M+Na]^+$ ($C_{14}H_{11}O_3INa$) required *m/z* 376.96451; found *m/z* 376.96436.

c) 2,3-Bis(benzyloxy)-5-iodo-benzaldehyde (7)



In acetone (20 mL) were suspended **6** (3.8 g, 10.7 mmol, 1.0 eq.) and anhyd. potassium carbonate (1.6 g, 11.8 mmol, 1.1 eq.), benzyl bromide (1.4 mL, 2.0 g, 11.8 mmol, 1.1 eq.) was added and reaction mixture was stirred on reflux 2 hours. After completion of the reaction[7], reaction mixture was diluted with ether (200 mL) and filtered. Filtrate was evaporated and obtained crude product was triturated with pentane, then crystalized from warm abs. ethanol (65°C) and dried on vacuum to give 4.1 g of pure product **7** (9.3 mmol, 87%) as a white solid.

^1H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 10.00 (s, 1H, C*H*O), 7.63 (d, 1H, J=2.1Hz, 3-*H*), 7.42 (d, 1H, J=2.1Hz, 13-*H*), 7.40-7.31 (m, 5H, Ph-*H*), 7.24-7.17 (m, 5H, Ph-*H*), 5.08 (s, 2H, 13-*H*), 5.06 (s, 2H, 8-*H*).

$^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ (ppm) = 188.73 (C-1), 153.12 (C-6), 151.48 (C-7), 136.01, 135.77 (both q-Ph*C)*, 131.80, 129.03, 129.00, 128.92, 128.85, 128.75, 128.72, 128.33, 127.90 (all Ph*C*H), 87.28 (C-4), 76.66 (C-8), 71.76 (C-13).

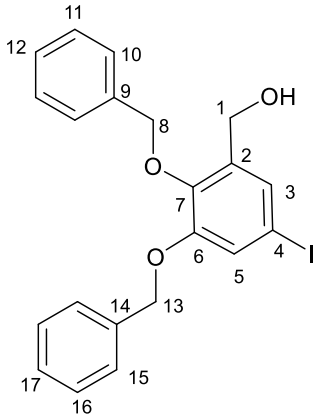
TLC (silica, EtOAc / cyclohex. 1:4 v/v, det.: UV_{254nm} & Stain A): R_f = 0.62

mp 112 °C

HRMS (ESI+): exact mass calculated for $[M+Na]^+$ ($C_{21}H_{17}O_3INa$) required *m/z* 467.01146; found *m/z* 467.01123.

[7] tracking by TLC (silica, EtOAc / cyclohex. 1:4 v/v, det.: UV_{254nm} & Stain A)

d) (2,3-bis(benzyloxy)-5-iodophenyl)methanol (8)



The aldehyde **7** (4.0 g, 9.0 mmol, 1.0 eq.) was suspended in MeOH (300 mL), suspension was cooled on ice bath and over 10 minutes, in small portions, sodium borohydride (170 mg, 4.5 mmol, 0.5 eq.) was added. Reaction mixture was allowed to warm up to room temperature and stirred until completion of the reaction[8] (about 3 hours). Solvent was evaporated, residue was dissolved in DCM[9] (200 mL) and washed with 0.1M HCl_{aq} (50 mL), water (30 mL) and brine. Organic layer was dried over anhyd. $MgSO_4$. The solvent was removed under reduced pressure to give 3.9 g of pure product **8** (8.6 mmol, 96%) as a yellow solid.

^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) = 7.39-7.26 (m, 10H, Ph*H*), 7.22 (d, 1H, J=2.1Hz, 3-*H*), 7.20 (d, 1H, J=2.1Hz, 5-*H*), 5.02 (s, 2H, 13-*H*), 4.98 (s, 2H, 8-*H*), 4.40 (s, 2H, 1-*H*).

^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$): δ (ppm) = 152.49 (C-6), 146.07 (C-7), 137.37 (C-2), 137.18 (C-9), 136.36 (C-14), 130.28 (C-3), 128.88, 128.84, 128.76, 128.61, 128.50, 127.84 (all Ph-*C*H), 123.05 (C-5), 87.12 (C-4), 75.35 (C-8), 71.49 (C-13), 60.91 (C-1).

TLC (silica, EtOAc / cyclohex. 1:4 v/v, det.: UV_{254nm} & Stain A): R_f = 0.34

mp 121 °C

HRMS (ESI+): exact mass calculated for $[M+Na]^+$ ($C_{21}H_{19}O_3INa$) required *m/z* 469.02711; found *m/z* 469.02662.

2,3-Bis(benzyloxy)-5-iodobenzyl acetate (acetylated derivative of **8**) was formed as significant by-product when EtOAc was used as a solvent instead of DCM:

TLC (silica, EtOAc / cyclohex. 1:4 v/v, det.: UV_{254nm} & Stain A): R_f = 0.72

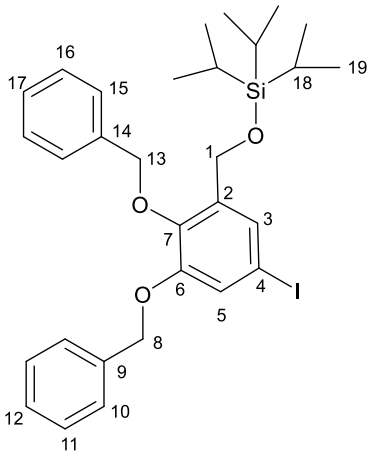
mp 114 °C

HRMS (ESI+): exact mass calculated for $[M+Na]^+$ ($C_{23}H_{21}O_4INa$) required *m/z* 511.03767; found *m/z* 511.03716.

[8] tracking by TLC (silica, EtOAc / cyclohex. 1:4 v/v, det.: UV_{254nm} & Stain A)

[9] by using EtOAc, acetylated by-product was formed (transesterification)

e) ((2,3-bis(benzyloxy)-5-iodobenzyl)oxy)triisopropylsilane (9)



The alcohol **8** (9.3 g, 20.8 mmol, 1.0 eq.) was dissolved in DCM (200 mL), imidazole (5.9 g, 86.7 mmol, 4.2 eq.) and triisopropylsilyl chloride (10.4 mL, 48.8 mmol, 2.3 eq.) were added. Reaction mixture was stirred at room temperature overnight, then diluted with DCM (200 mL) and washed with water (100 mL), 5% aq. sol. citric acid (2 x 100 mL) and brine. The solvent was removed under reduced pressure and residual crude product was dissolved in cyclohexane and purified by filtration through short Alox column. Alox was washed with cyclohexane and combined filtrates were evaporated to give 12.1 g of pure product **9** (20.1 mmol, 96%) as a colourless oil.

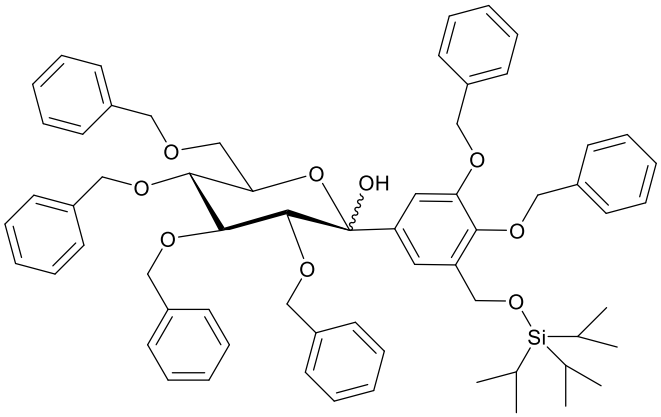
^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.42-7.27, 7.22 (m, 10H, Ph*H*), 7.16 (d, 1H, J=2Hz), 5.02 (s, 2H, 3-*H*), 4.92 (s, 2H, 5-*H*), 4.58 (s, 2H, 1-*H*), 1.04-0.95 (m, 21H)

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ (ppm) = 152.06 (C-6), 144.68 (C-7), 138.39, 137.60, 136.60 (C-3, C-9, C-14), 128.86, 128.83, 128.73, 128.63, 128.39, 128.36, 127.80 (C-2, C-10, C-11, C-12, C-15, C-16, C-17), 121.94 (C-5), 87.20 (C-4), 75.01 (C-13), 71.40 (C-8), 60.16 (C-1), 18.22 (C-19), 12.11 (C-18).

TLC (silica, EtOAc / cyclohex. 1:4 v/v, det.: UV_{254nm} & Stain A): R_f = 0.89

HRMS (ESI+): exact mass calculated for $[M+Na]^+$ ($C_{30}H_{39}O_3ISiNa$) required *m/z* 625.16054; found *m/z* 625.16046.

f) (3R,5R,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-2-(3,4-bis(benzyloxy)-5-(((triisopropylsilyl)oxy)methyl)phenyl)tetrahydro-2H-pyran-2-ol (10)



The iodide **9** (5.0 g, 8.3 mmol, 1.0 eq.) was dissolved in mixture anhydr. THF / dry toluene[10] 1:4 v/v (125 mL) and the solution was cooled on -78°C. Under an inert atmosphere 2.7M n-butyllithium solution in heptane (3.4 mL, 9.1 mmol, 1.1 eq.) was added dropwise and stirring was continued with cooling. After 30 minutes a solution of 2,3,4,6-Tetra-*O*-benzyl-D-glucono-1,5-lactone (13.4 g, 24.9 mmol, 3.0 eq.) in dry toluene[10] (50 mL) was added dropwise and reaction mixture was further stirred for next 1 hour at -78°C. At the same temperature glac. AcOH (15.2 mL, 265.8 mmol, 32 eq.) was added dropwise and 10 minutes later slowly was added sat. aq. sol. $NaHCO_3$ (121 mL) and then reaction mixture was

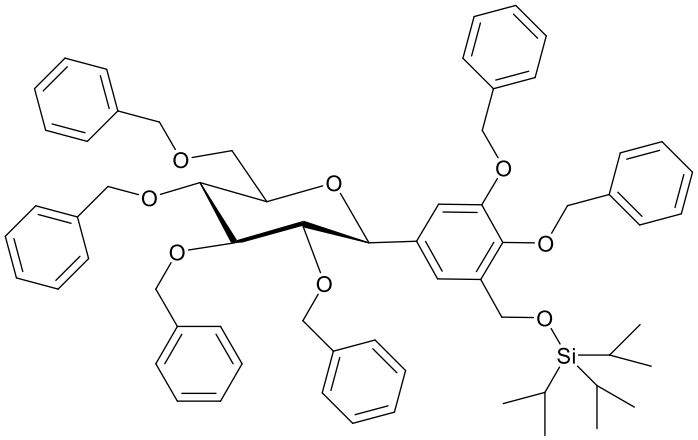
[10] dried over sodium pieces

warmed up to room temperature. Resulting mixture was extracted with EtOAc (2 x 150 mL). Combined organic layers were washed with brine and dried over anhyd. $MgSO_4$. The solvent was removed under reduced pressure and the residual crude product **10** (17.3 g) in form of colourless to pale yellow oil was used as is for the next step of synthesis. [11]

TLC (silica, EtOAc / cyclohex. 1:2 v/v, det.: UV_{254nm} & Stain A): R_f = 0.66

HRMS (ESI+): exact mass calculated for $[M+Na]^+$ ($C_{64}H_{74}O_9SiNa$) required *m/z* 1027.49943; found *m/z* 1037.49929.

g) ((2,3-bis(benzyloxy)-5-((2S,3S,5R,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-tetrahydro-2H-pyran-2-yl)benzyl)oxy)triisopropylsilane (11)



The crude hemiketal **10** (17.1 g, 16.9 mmol, 1.0 eq.) was dissolved in dry DCM[12] (300 mL). In inert atmosphere and with cooling on -78°C, triethylsilane (8.1 mL, 50.6 mmol, 3.0 eq.) followed by boron trifluoride diethyl etherate (6.2 mL, 50.6 mmol, 3.0 eq.) were added over 10 minutes. Reaction mixture was stirred at -78°C for 45 minutes, then at -30°C for 5 minutes and after that the reaction was quenched at the same temperature by adding sat. aq. sol. $NaHCO_3$ (350 mL). Reaction mixture was left with intensive stirring to warm up to room temperature. Layers were separated and aqueous layer was extracted with DCM (100 mL). Combined organic layers were washed with brine and dried over anhyd. $MgSO_4$. The solvent was removed under reduced pressure and the residual crude product **11** (17.0 g) in form of pale yellow oil was used as is for the next step of synthesis.

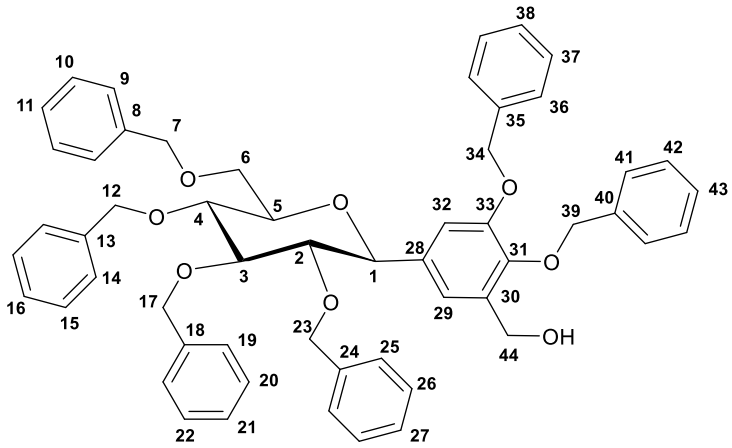
TLC (silica, EtOAc / cyclohex. 1:4 v/v, det.: UV_{254nm} & Stain A): R_f = 0.62 & 0.67

MALDI-ToF MS (DHB, EtOAc): mass calculated for $[M+Na]^+$ ($C_{64}H_{74}O_8SiNa$) required *m/z* 1021.50; found *m/z* 1021.48.

[11] It should be used for a next step in few days. Stability test showed that this compound is not stable for a longer period of time.

[12] dried overnight over molecular sieves 4Å

h) (2,3-bis(benzyloxy)-5-((2S,3S,5R,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-tetrahydro-2H-pyran-2-yl)phenyl)methanol (12)



The crude product **11** (16.5 g, 16.5 mmol, 1.0 eq.) was dissolved in EtOH (150 mL), conc. hydrochloric acid (8.3 mL, 99.1 mmol, 6.0 eq.) was added and reaction mixture was stirred for 1 hour at 50°C and then at room temperature overnight. After completion of the reaction[13], solid $NaHCO_3$ was added in small portions until neutral pH and obtained suspension was evaporated. Residual solid was suspended in DCM (250 mL), stirred and filtered. Filtrate was washed with water (2 x 50 mL) and brine and dried over anhyd. $MgSO_4$. The solvent was removed under reduced pressure and residual white waxy solid was triturated with pentane (2 x 100 mL) and then precipitated from ether/pentane[14]. Obtained solid was filtered on sinter and dried on high vacuum to give 3.3 g of pure product **12** (3.9 mmol, 47%[15]) as a white solid.

^{1}H NMR (600MHz, $CDCl_3$): δ = 7.36-7.10 and 6.87-6.86 (m, 30H, Ph*H*), 6.99 (s, 1H, 32-*H*), 6.95 (s, 1H, 29-*H*), 5.04 (d, 1H, *J*=10.9Hz, 34-*H*α), 5.01 (d, 1H, *J*=10.9Hz, 34-*H*β), 4.95 (s, 2H, 44-*H*), 4.89 (d, 1H, *J*=11.1Hz, 39-*H*α), 4.83 (d, 1H, *J*=11.1Hz, 39-*H*β), 4.79 (d, 1H, *J*=10.7Hz, Ph-C*H*$_{2\ \alpha/\beta}$), 4.58-4.55 (m, 2H, Ph-C*H*$_{2\ \alpha/\beta}$), 4.50-4.46 (m, 3H, Ph-C*H*$_{2\ \alpha/\beta,}$ 6-*H*α), 4.34 (d, 1H, 10.3Hz, Ph-C*H*$_{2\ \alpha/\beta}$), 4.15 (d, 1H, *J*=9.5Hz, 1-*H*), 3.74-3.66 (m, 5H, 3-*H*, 4-*H*, 6-*H*β 2Ph-C*H*$_{2\ \alpha/\beta}$), 3.52-3.50 (m, 1H, 5-*H*), 3.36 (m, 1H, 2-*H*).

^{13}C{^{1}H} NMR (150MHz, $CDCl_3$): δ = 151.50 (C-31), 145.79 (C-33), 138.83 (C-40), 138.45 (q-Ph*C*), 138.31 (q-Ph*C*), 137.83 (q-Ph*C*), 137.37 (C-35), 136.84 (q-Ph*C*), 135.36 (C-28), 135.12 (q-Ph*C*), 128.82, 128.69, 128.67, 128.62, 128.55, 128.55, 128.50, 128.42, 128.37, 128.31, 128.17, 128.13, 127.89, 127.85, 127.82, 127.80, 127.73, 127.72, 127.70 (all Ph*C*H and C-30), 120.40 (C-29), 113.00 (C-32), 86.77 (C-3), 84.43 (C-2), 81.45 (C-1), 79.43 (C-5), 78.39 (C-4), 77.37 (Ph-*C*H_2), 77.16 (Ph-*C*H_2), 76.95 (Ph-*C*H_2), 75.75 (C-39), 75.25 (C-34), 75.25 (Ph-*C*H_2), 74.96 (C-6), 73.57 (Ph-*C*H_2), 70.95 (C-44), 69.22 (Ph-*C*H_2), 61.77 (Ph-*C*H_2).

TLC (silica, EtOAc / cyclohex. 1:3 v/v, det.: UV_{254nm} & Stain A): R_f = 0.23

mp 151 °C

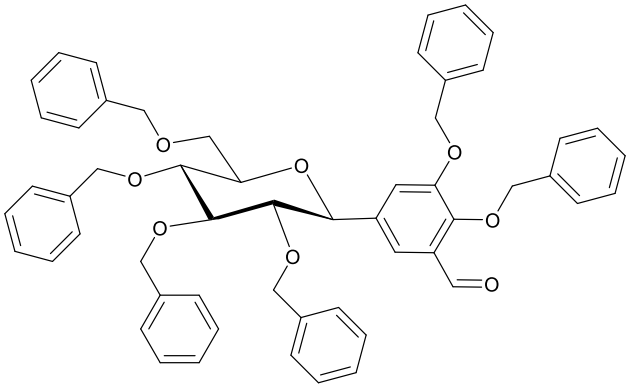
HRMS (ESI+): exact mass calculated for $[M+Na]^+$ ($C_{55}H_{54}O_8Na$) required *m/z* 865.37109; found *m/z* 865.37129.

[13] tracking by TLC (silica, EtOAc / cyclohex. 1:4 v/v, det.: UV_{254nm} & Stain A)

[14] Dissolved in minimal amount of ether and precipitated by slow addition of pentane in ultrasound bath. The procedure was repeated 3 times.

[15] over three steps

i) 2,3-bis(benzyloxy)-5-((2S,3S,5R,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-tetrahydro-2H-pyran-2-yl)benzaldehyde (13)

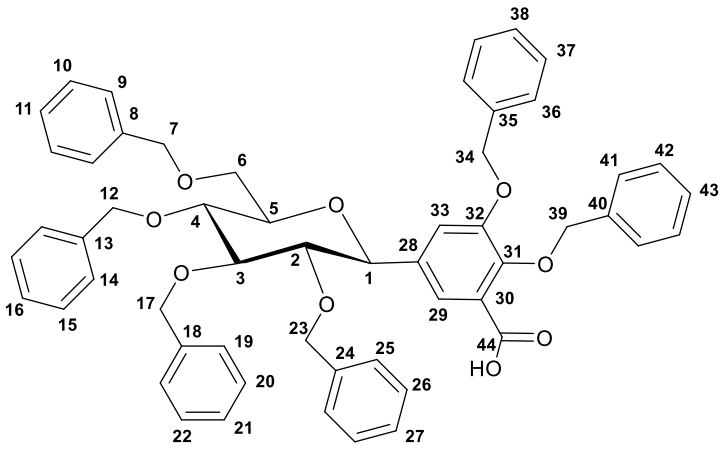


The alcohol **12** (2.2 g, 2.6 mmol, 1.0 eq.) was dissolved in DCM saturated with water[16] (200 mL), Dess–Martin periodinane (2.2 g, 5.2 mmol, 2.0 eq.) was added and the reaction mixture was vigorously stirred at room temperature overnight. After completion[17] the reaction mixture was evaporated at room temperature and residue was dissolved in ether (200 mL). Obtained solution was washed with the mixture sat.sol. $Na_2S_2O_3$ / sat.sol. $NaHCO_3$ 1:1 v/v (2 x 100 mL), water (50 mL) and brine. Organic layer was dried over anhyd. $MgSO_4$. The solvent was removed under reduced pressure and obtained crude product **13** (2.1 g) in form of white solid was used as is for the next step of synthesis.

TLC (silica, EtOAc / cyclohex. 1:3 v/v, det.: UV_{254nm} & Stain A): $R_f = 0.52$

HRMS (ESI+): exact mass calculated for $[M+Na]^+$ ($C_{55}H_{52}O_8Na$) required *m/z* 863.35544; found *m/z* 863.35503.

j) 2,3-bis(benzyloxy)-5-((2S,3S,5R,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-tetrahydro-2H-pyran-2-yl)benzoic acid (14)



The crude aldehyde **13** (2.1 g, 2.5 mmol, 1.0 eq.) was dissolved in dioxane (30 mL). Sulfamic acid (440 mg, 4.5 mmol, 1.8 eq.) was dissolved in water (8 mL) and added to the aldehyde solution. Separately, sodium chlorite[18] (410 mg, 4.5 mmol, 1.8 eq.) was dissolved in water (4 mL) and in a few portions, during 10 minutes, added to the reaction mixture. Stirring was continued at room temperature next 20 minutes and then reaction was partitioned between water (50 mL) and EtOAc (100 mL). Aqueous layer was extracted with EtOAc (2 x 50 mL). Combined organic layers were washed with brine and dried over anhyd.

[16] DCM was in separatory funnel shaken with several milliliters of water, then separated from water and used as is for the reaction

[17] tracking by TLC (silica, EtOAc / cyclohex. 1:3 v/v, det.: UV_{254nm} & Stain A)

[18] Caution: sodium chlorite is toxic oxidizing reagent

$MgSO_4$. The solvent was removed under reduced pressure and residual crude product was purified by crystallisation from MeOH / pentane solution to give 1.9 g of pure product **14** (2.2 mmol, 86%[19]) as a white solid.

^{1}H NMR (600MHz, $CDCl_3$): δ = 11.2 (broad s, 1H, COO*H*), 7.79 (d, 1H, 29-*H*), 7.34-7.11 and 6.87-6.86 (m, 31H, Ph*H*), 5.18 (d, 1H, *J*=10.4Hz, 34-H_α), 5.15 (d, 1H, *J*=10.4Hz, 34-H_β), 4.91 (d, 1H, *J*=11.0Hz, 39-H_α), 4.89 (d, 1H, *J*=11.0Hz, 39-H_β), 4.88 (d, 1H, *J*=11.2Hz, Ph-C$H_{2\ \alpha/\beta}$), 4.86 (d, 1H, *J*=11.2Hz, Ph-C$H_{2\ \alpha/\beta}$), 4.79 (d, 1H, *J*=10.7Hz, Ph-C$H_{2\ \alpha/\beta}$), 4.56 (m, 2H, Ph-C$H_{2\ \alpha/\beta}$, 6-H_α), 4.49 (d, 1H, *J*=12.3Hz, Ph-C$H_{2\ \alpha/\beta}$), 4.40 (d, 1H, *J*=10.7Hz, Ph-C$H_{2\ \alpha/\beta}$), 4.15 (d, 1H, *J*=9.5Hz, 1-*H*), 3.77-3.66 (m, 5H, 3-*H*, 4-*H*, 6-H_β, 2Ph-C$H_{2\ \alpha/\beta}$), 3.55-3.52 (m, 1H, 5-*H*), 3.35 (pseudo t, 1H, *J*=9.2Hz 2-*H*).

^{13}C NMR (150MHz, $CDCl_3$): δ = 165.07 (C-44), 151.09, 146.62, 138.71, 138.34, 138.18, 137.61, 136.58, 135.91, 134.69 (all q-Ph*C*), 129.45, 129.42, 128.94, 128.90, 128.66, 128.58, 128.57, 128.53, 128.39, 128.26, 128.15, 127.96, 127.95, 127.89, 127.83, 127.79, 127.76, 127.74, 123.36, 122.88 (all Ph-*C*H), 118.02 (C-30), 86.87 (C-3), 83.86 (C-2), 80.74 (C-1), 79.49 (C-5), 78.40 (C-4), 76.95 (Ph-*C*H_2), 75.74 (Ph-*C*H_2), 75.28 (Ph-*C*H_2), 75.07 (C-6), 73.57 (Ph-*C*H_2), 71.46 (Ph-*C*H_2), 69.20 (Ph-*C*H_2).

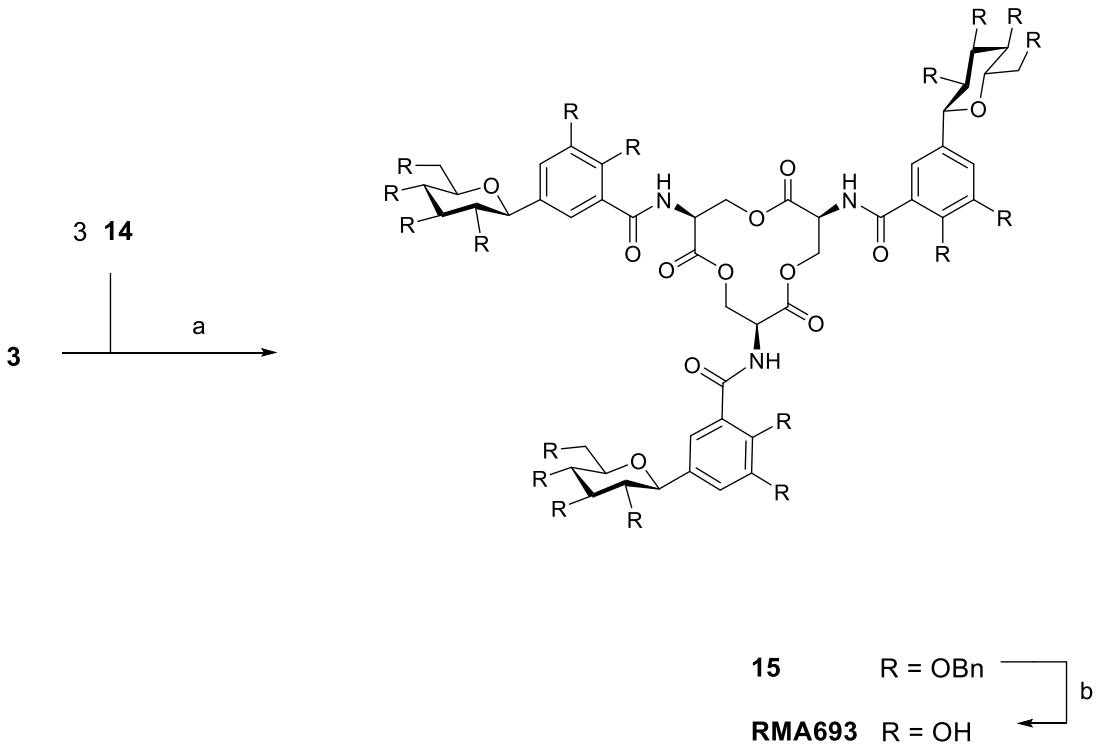
TLC (silica, EtOAc / cyclohex. 1:1 v/v, det.: UV_{254nm} & Stain A): R_f = 0.50 (spot with tailing)

mp 96 °C

HRMS (ESI-): exact mass calculated for $[M-H]^-$ ($C_{55}H_{51}O_9$) required *m/z* 855.35386; found *m/z* 855.35302.

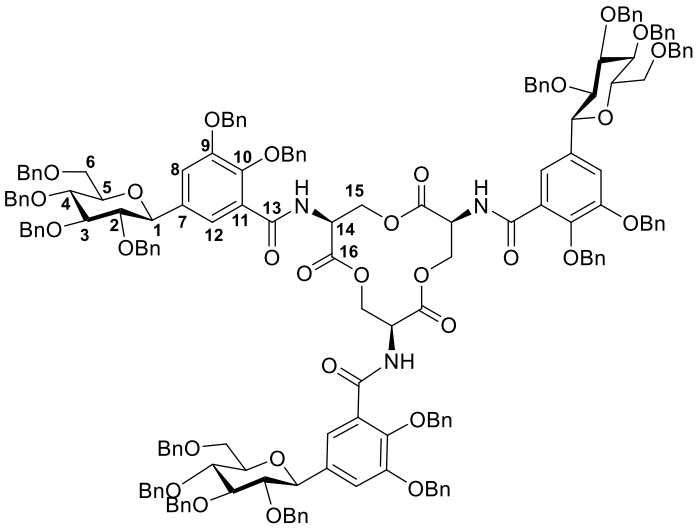
[19] over two steps

2.3 Preparation of RMA693 (tri-glucosylated enterobactin)



Scheme S3: Synthesis of RMA693. Reagents and conditions: (a) PyAOP, DIPEA, ACN, rt ; (b) H_2, $Pd(OH)_2/C$, THF/H_2O 1:1

a) Perbenzylated RMA693 (15)



The lactone **3** (100 mg, 271 µmol, 1.0 eq.) was dissolved in ACN (2 mL), DIPEA (425 µL, 2.4 mmol, 9.0 eq.) was added and the mixture was stirred 10 minutes at room temperature. The acid **14** (696 mg, 813 µmol, 3.0 eq.) and PyAOP (440 mg, 846 µmol, 3.1 eq.) were separately dissolved in ACN (10 mL), obtained solution was stirred at room temperature for 10 minutes and then added to the reaction mixture with lactone. Reaction mixture was stirred at room temperature overnight and then evaporated[20]. Residual yellow oil was partitioned between ether (100 mL) and water (50 mL). Aqueous layer was extracted with ether (100 mL). Combined organic layers were washed with 5% aq. citric acid (50 mL), sat. aq. sol. $NaHCO_3$ (50 mL) and brine and dried over anhyd. $MgSO_4$. The solvent was removed under reduced pressure. Residual crude product was dissolved in DCM, loaded on dry silica (30 g) and dried

[20] With heating on 35°C

on vacuum. The crude product absorbed on silica was washed on sinter with DCM (200 mL) and mixture cyclohex. / EtOAc 4:1 v/v (1000 mL)[21] and after that the product was extracted[22] from silica with 30% EtOAc/cyclohex. Collected extract solution was evaporate under reduced pressure to give 578 mg of pure product **15** (208 µmol, 77%) as a white solid foam.

^{1}H NMR (600MHz, $CDCl_3$): δ = 8.44 (d, 3H, *J*=7.4Hz, N*H*) 7.78 (s, 3H, 12-*H*), 7.30-7.07 and 6.88-6.86 (m, 93H, Ph*H*), 5.11 (d, 3H, *J*=10.8Hz, Ph-C$H_{2\ \alpha/\beta}$), 4.98 (d, 3H, *J*=10.8Hz, Ph-C$H_{2\ \alpha/\beta}$), 4.95-4.91 (m, 3H, 14-*H*), 4.87-4.77 (m, 15H, Ph-C$H_{2\ \alpha/\beta}$), 4.56-4.53 (m, 6H, Ph-C$H_{2\ \alpha/\beta}$, 6-H_α), 4.48 (d, 3H, *J*=12.2Hz, Ph-C$H_{2\ \alpha/\beta}$), 4.33 (d, 3H, *J*=10.5Hz, Ph-C$H_{2\ \alpha/\beta}$), 4.15 (d, 3H, 9.4Hz, 1-*H*), 4.09-4.07 (m, 3H, 15-H_α), 4.04-4.01 (m, 3H, 15-H_β), 3.73-3.64 (m, 15H, 3-*H*, 4-*H*, 6-H_β 2Ph-C$H_{2\ \alpha/\beta}$), 3.53-3.51 (m, 3H, 5-*H*), 3.36 (pseudo t, 3H, *J*=9.2Hz, 2-*H*),

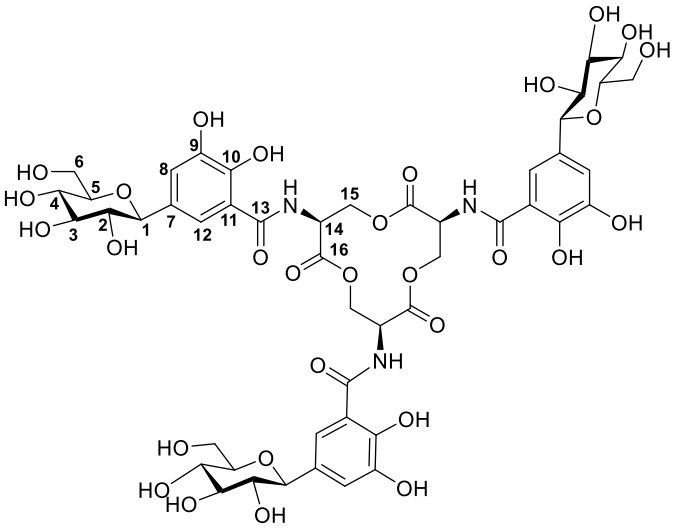
^{13}C NMR (150MHz, $CDCl_3$): δ = 169.43 (C-16), 164.56 (C-13), 151.38, 146.52, 138.72, 138.31, 138.16, 137.63, 136.19, 135.83, 135.58 (all q-Ph*C*), 129.07, 128.77, 128.66, 128.54, 128.43, 128.42, 128.39, 128.27, 128.25, 128.20, 128.18, 128.03, 127.78, 127.76, 127.74, 127.73, 127.66, 127.58, 126.02, 125.53, 122.29, 116.60 (all Ph*C*H and C-11), 86.72 (C-3), 83.97 (C-2), 80.95 (C-1), 79.39 (C-5), 78.33 (C-4), 76.37 (Ph-*C*H_2), 75.57 (Ph-*C*H_2), 75.13 (Ph-*C*H_2), 74.89 (C-6), 73.46 (Ph-*C*H_2), 71.14 (Ph-*C*H_2), 69.16 (Ph-*C*H_2), 64.05 (C-15), 51.28 (C-14).

TLC (silica, EtOAc / cyclohex. 1:2 v/v, det.: UV_{254nm} & Stain A): R_f = 0.32 (spot with tailing)

mp 89 °C

MS-MALDI (DHB, EtOAc): mass calculated for $[M+Na]^+$ ($C_{174}H_{165}N_3O_{30}Na$) required *m/z* 2799.14; found *m/z* 2799.25.

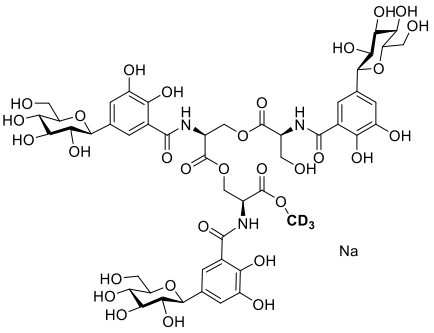
b) RMA693



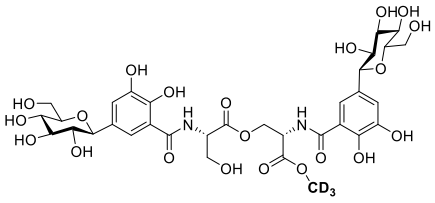
The compound **15** (300 mg, 108 µmol, 1.0 eq.) was dissolved in THF (5 mL), deionised water[23] (5 mL) and catalytic amount of palladium hydroxide on activated charcoal (20% Pd) were added. Reaction mixture was degassed under vacuum and stirred overnight at room temperature connected to a double layer balloon of hydrogen. The catalyst was removed by filtration and the filtrate was evaporate under reduced pressure to give 125 mg of pure product **RMA693** (108 µmol, 100%) as a white solid.

[21] to remove impurities
[22] silica was washed on sinter with the solvent and the extract solution was collected
[23] Water for ionic chromatography was used

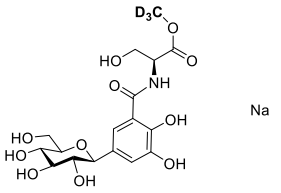
No evaluable ^{1}H or ^{13}C NMR could be obtained in D_2O. In MeOD degradation was detected over time (see also chapter 2.4). The corresponding degradation products were identified by MALDI-MS-spectra (DHB, H_2O/CH_3CN) as:



Chemical Formula: $C_{49}H_{58}D_3N_3NaO_{31}$
Exact Mass: 1213,338



Chemical Formula: $C_{33}H_{39}D_3N_2NaO_{21}$
Exact Mass: 828,237



Chemical Formula: $C_{17}H_{20}D_3NNaO_{11}$
Exact Mass: 443,136

For identification and characterization of **RMA693** a small amount was solubilized in d_4-MeOD directly before measurement:

^{1}H NMR (500MHz, d_4-MeOD): δ = 7.39 (d, 1H, *J*=2.0Hz, 12-*H*), 7.05 (d, 1H, *J*=2.0Hz, 8-*H*), 5.05 (dd, 1H, *J*=6.4Hz, 4.3Hz, 14-*H*), 4.68 (dd, 1H, *J*=11.1Hz, 6.4Hz, 15-H_α), 4.59 (dd, 1H, *J*=11.1Hz, 4.3Hz, 15-H_β), 4.03 (d, 1H, *J*=9.3Hz, 1-*H*), 3.89-3.85 (m, 1H, 6-H_β), 3.72-3.68 (m, 1H, 6-H_α), 3.47-3.35 (m, 4H, 2,3,4,5-*H*).

^{13}C NMR (125MHz, d_4-MeOD): δ = 170.81 (C-16), 170.66 (C-13), 149.43, 146.88, 131.51, 119.71 (C-8), 119.30 (C-12), 116.20 (C-11), 83.02 (C-1), 81.94, 79.64, 76.28, 71.79 (C-2,3,4,5), 65.87 (C-15), 63.02 (C-6), 53.53 (C-14).

TLC (silica, ACN / H_2O 4:1 v/v, det.: UV_{254nm}, I_2 & stain A): R_f = 0.39

mp 185 °C (decomp.)

HRMS (ESI+): exact mass calculated for $[M+Na]^+$ ($C_{48}H_{57}N_3O_{30}Na$) required *m/z* 1178.29191; found *m/z* 1178.29236.

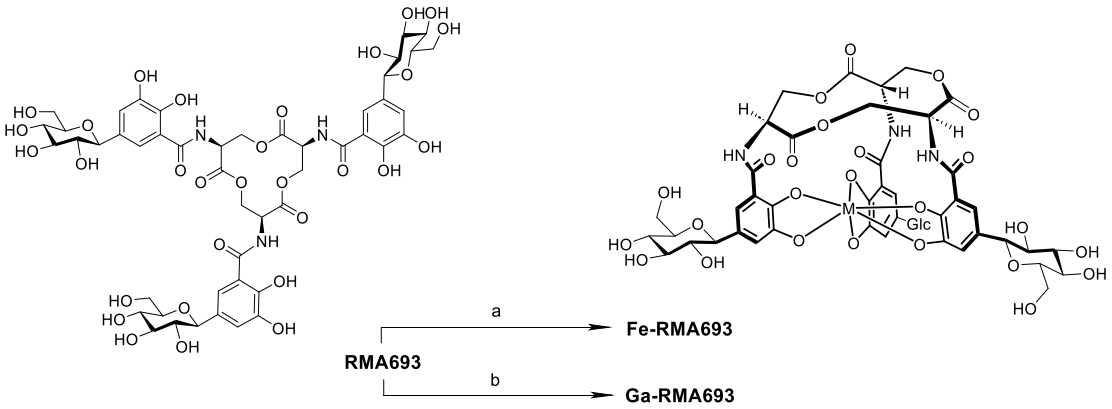
2.4 Stability of RMA693

Due to the very small amount of compound used for labeling experiments, a stock solution of the ligand **RMA693** should be prepared and it is desirable that the stock solution is stable for a longer period of time. Consequently, the stability of the compound in solution is a very important and must be taken into account. During the work on synthesis of **RMA693** it was observed that the compound is pretty sensitive to nucleophilic degradation. Other authors previously observed a similar[24] sensitivity during their work on enterobactin where the sensitivity was attributed to the macrolactone ring, which is the same for both compounds. Therefore, the stability of **RMA693** was additionally studied by re-measurement of NMR

[24] T. Zheng, J.L. Bullock, and E.M. Nolan, *J. of the Am. Chem. Soc.*, 2012, **134**, 18388-18400.

spectra from earlier prepared and measured solutions of the compound in deuterated solvents or by monitoring the stability of the solutions in non-deuterated solvents using TLC and MALDI-MS (see also chapter before). Because of the high polarity of **RMA693**, the choice of solvents is limited to very polar and mostly protic solvents. It was found that **RMA693** is not stable in acidic and basic conditions, in contact with nucleophiles or oxidizing agents as well as in MeOH or H_2O solution[25], but it is stable in D_2O solution, also at room temperature and for longer time periods[26]. Sensitivity on UV/Vis light was not observed. The big difference in the stability of **RMA693** in H_2O and D_2O solutions could be explained by the influence of microorganisms in the non-deuterated water on the one side and deuterium kinetic isotope effect[27] and his influence on toxicity of D_2O (in higher concentrations) for living organisms[28] on the other side. Stability in sterilized non-deuterated water has not been studied. Thanks to the appropriate stability, we used D_2O stock solution of **RMA693** for all our radiolabeling experiments. Due to the high dilution, the concentration of D_2O in the final sample is negligibly low and without any biological effect.

2.5 Preparation of cold reference Fe-RMA693 and Ga-RMA693



Scheme S4: Metal complexes of salmochelin. Reagents and conditions:
(a) $Fe(TMHD)_3$, MeOH/EtOAc 1:1, rt ; (b) $Ga(TMHD)_3$, MeOH/EtOAc 1:1, rt

a) Fe-RMA693

RMA693 (10 mg, 8.6 umol, 1.0 eq.) was dissolved in MeOH (0.5 mL). Separately was tris(2,2,6,6-tetramethyl-3,5-heptanedionato)iron(III) (6.3 mg, 10.3 µmol, 1.2 eq.) dissolved in EtOAc (0.5 mL) and that solution was added to **RMA693** solution. Reaction mixture was stirred overnight at room temperature and then evaporated[29]. Residual solid was triturated with

[25] for longer than few days
[26] at least one year
[27] D.J. Kushner, A. Baker, and T.G. Dunstall, *Can. J. Physiol. Pharmacol.*, 1999, **77**, 79-88; A.J. Kresge, *Pure Appl. Chem.*, 1964, **8**, 243-258; X. Zhou, L. Wang, X. Fan, B. Wilfong, S.C. Liou, Y. Wang, H. Zheng, Z. Feng, C. Wang, and E.E. Rodriguez, *Chem. Mater.*, 2020, **32**, 769-775.
[28] P.M. Misra, *Curr. Sci.*, 1967, **36**, 447–453; O. Mosin, I. Ignatov, *Eur. Rev. Chem. Res.*, 2015, **3**, 25-42; J.F. Thomson, *Ann. N.Y. Acad. Sci.*, 1960, **84**, 736–744.
[29] With heating on 35°C

pentane (3 x 20 mL) and EtOAc (20mL) and dried on high vacuum to give 10 mg of product **Fe-RMA693** (8.3 µmol, 97%) as a purple solid.

TLC (silica, ACN / H_2O 4:1 v/v, det.: UV/Vis, Stain C): R_f = 0.50

HRMS (NSI-): exact mass calculated for [M+H] (*z*=2) ($C_{48}H_{52}N_3O_{30}Fe^{2-}$) required *m/z* 603.09987; found *m/z* 603.10025.

b) Ga-RMA693

RMA693 (10 mg, 8.6 umol, 1.0 eq.) was dissolved in MeOH (0.5 mL). Separately was tris(2,2,6,6-tetramethyl-3,5-heptanedionato)gallium(III) (6.4 mg, 10.3 µmol, 1.2 eq.) dissolved in EtOAc (0.5 mL) and that solution was added to TGE solution. Reaction mixture was stirred overnight at room temperature and then evaporated[30]. Residual solid was triturated with pentane (3 x 20 mL) and EtOAc (20mL) and dried on high vacuum to give 10 mg of product **Ga-RMA693** (8.2 µmol, 95%) as a white solid.

TLC (silica, ACN / H_2O 4:1 v/v, det.: UV_{254nm}, I_2, Stain A): R_f = 0.24

HRMS (NSI-): exact mass calculated for [M+H] (*z*=2) ($C_{48}H_{52}N_3O_{30}Ga^{2-}$) required *m/z* 609.59512; found *m/z* 609.59460.

3. Radiolabelling of RMA693 (manually)

3.1 Preparation of radiolabelled salmochelin [^{68}Ga]Ga-RMA693

^{68}Ga was eluted from ^{68}Ge/^{68}Ga generator by fractional elution[31] with 0.1M HCl (5 mL). Obtained generator eluate (1 mL, 350-600 MBq of $^{68}GaCl_3$) was diluted with PBS-buffer pH=7.4 (200 uL), neutralised with a few[32] drops 1M $NaHCO_3$ and then mixed with stock solution of **RMA693** in deuterium oxide[33] (16ug/uL, 8 uL). Reaction mixture was incubated on 50°C for 10 minutes and filtered through PTFE filter 0.2 um. Radiochemical purity of obtained labeled product **[^{68}Ga]Ga-RMA693** was analyzed and the tracer was used as is for further experiments.

[30] with heating on 35°C

[31] During elution from the generator 5 fractions of 1 mL were collected. Only fraction with the highest activity (usually 3. fraction) was used for labeling

[32] Until pH=7.4 (about 8 - 10 drops)

[33] See the chapter 2.4

3.2 Radiochemical purity

Radiochemical purity of labeled product **[^{68}Ga]Ga-RMA693** was analyzed on radio-HPLC, by previously described method, as well as on TLC[34] using HILIC[35] conditions on silica impregnated plates, scanned after developing on gamma TLC plate scanner.

The retention factor (R_f) of **[^{68}Ga]Ga-RMA693** on TLC was 0.4-0.5 (Fig. S1.b) and R_f of free ^{68}Ga (blank activity before complexation[36]) was 0.0 (Fig. S1.a). Radiochemical purity of **[^{68}Ga]Ga-RMA693** by this method was 99.6% and the only observed radioactive impurity was unreacted free ^{68}Ga (0.4%).

The retention time of **[^{68}Ga]Ga-RMA693** on radio-HPLC with previously described method was about 6 minutes and radiochemical purity by this method was 99.1%. (Fig. S1.d)

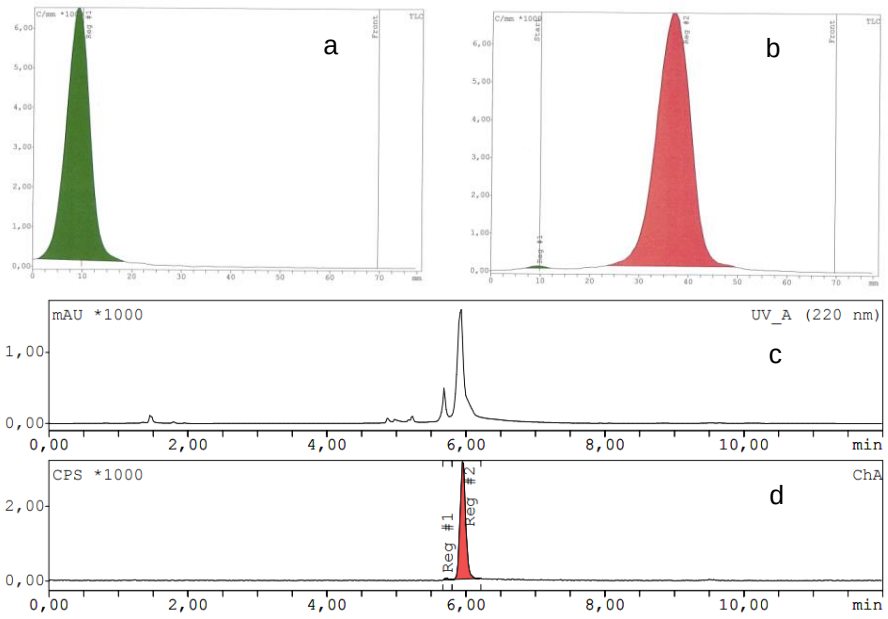


Fig.S1: Determination of radiochemical purity. Chromatograms: (a) TLC of the free ^{68}Ga activity[34]; (b) TLC of **[^{68}Ga]Ga-RMA693**; (c) HPLC of **[^{68}Ga]Ga-RMA-693** on UV detector (λ=220nm); (d) HPLC of **[^{68}Ga]Ga-RMA693** on gamma detector

3.3 *In vitro* characterisation of [^{68}Ga]Ga-RMA693

a) *Log P* value

Labeled product **[^{68}Ga]Ga-RMA693**, prepared by previously described method, was diluted with PBS-buffer to have solution (1 mL) of activity about 10 MBq. From this solution, a sample (10 µL) was taken and diluted further with PBS buffer (490 µL) and 1-octanol (500 µL). As a control, three tests were performed in parallel. Tubes with the mixtures were vigorously vortexed and centrifuged. From every of 3 extraction mixtures, sample of 1-octanol layer[37]

[34] TLC (SiO_2, ACN / H_2O 4:1 v/v)
[35] hydrophilic interaction liquid chromatography
[36] Prepared in the same way as the labeled compound **[^{68}Ga]Ga-RMA693**, but without adding the ligand
[37] upper layer

(400 µL) was taken and mixed with fresh amount of PBS-buffer (400 µL) and again vigorously vortexed and centrifuged. From every of that second extraction mixtures 3 samples of 1-octanol layer (3 x 100µL) and also 3 samples of PBS-buffer layer (3 x 100µL) were used for measurement. Obtained 9+9 samples were then immediately counted in a gamma-counter. The partition coefficient value was then calculated[38] from obtained decay corrected data as a mean value.

Measured *logP* value of **[^{68}Ga]Ga-RMA693** was 0.0060 (SD = 0.09).

b) Serum stability

The serum stability of **[^{68}Ga]Ga-RMA693** was tested by incubation of pure product (about 10 MBq) in human as well as in mouse serum (0.1 - 0.5 mL) at 37°C up to 120 minutes[39]. After incubation, proteins in human or mouse serum samples (20 uL) were precipitated with mixture DCM / MeOH 1:1 v/v (100 uL) and centrifuged. Degradation of complex **[^{68}Ga]Ga-RMA693** was evaluated by radio-HPLC directly injecting the liquid phase of precipitated samples and using the same method as previously described.

[^{68}Ga]Ga-RMA693 has been shown to remain stable in both human and murine serum up to 120 minutes.

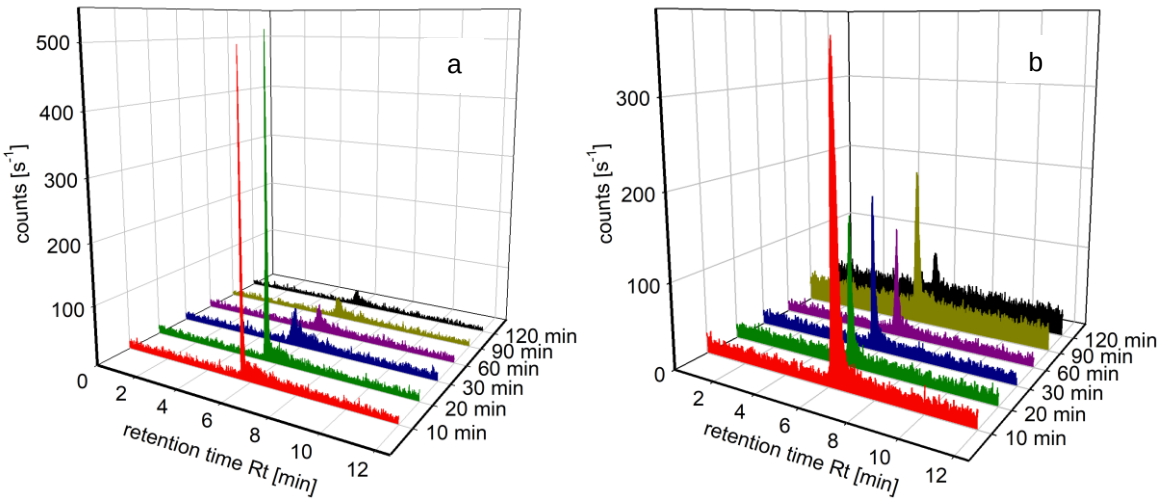


Fig.S2: Determination of serum stability. HPLC Chromatograms:
(a) murine blood serum stability test; (b) human blood serum stability test

[38] *logP* = log(counts per minute in 1-octanol layer / counts per minute in PBS-buffer layer)
[39] samples were taken and analyzed after 10, 20, 30, 60, 90 and 120 minutes

4. *In vitro* assay (radiotracer uptake)

Preparation of iron-depleted cation-adjusted Mueller Hinton Broth (ID-CAMHB)

ID-CAMHB was prepared following the protocol by Hackel et al.[40] In brief, 100g of Chelex® 100 resin (Bio-Rad Laboratories, Hercules, CA) was added to 1 L of Mueller-Hinton-broth (MHB) and the suspension stirred for 2 h at room temperature to remove cations in the medium. Then, after a short period of sedimentation of the resin, the medium was filtered through a 0.2 µm filter to remove the remaining resin. The pH was adjusted to 7.3 with 0.1 M hydrochloric acid and the medium was supplemented with 22.5 µg/mL calcium ($CaCl_2$), 11.25 µg/mL magnesium ($MgCl_2$), and 10 µM zinc ($ZnSO_4$). Finally, the medium was sterile-filtered using a 0.2 µm filter. The ID-CAMHB was now expected to contain ≤0.03 µg/mL iron.

in vitro uptake of [^{68}Ga]Ga-RMA693

Following bacterial strains were used in this study: *Escherichia coli* reference strain ATCC25922, *E. coli* laboratory strain TG1 (an *E. coli* K-12 derivate), and *S. aureus* mouse strain LS1.[41]

Bacteria were cultivated overnight in MHB at 37°C under shaking conditions. Next morning bacteria were adjusted to an optical density at 600 nm (OD_{600}) of 0.1 in MHB and further cultivated under shaking conditions at 37°C until the culture reached OD_{600} of 0.3. After a washing step with phosphate buffered saline (PBS) bacteria were re-suspended in MHB or in ID-CAMHB. The bacterial solution was incubated with 0.1 or 1 MBq/mL **[^{68}Ga]Ga-RMA693** and transferred into 6 well plates (2 mL/ well). The bacteria were incubated at 37 °C under shaking conditions. At designated time points (30 min, 1h, 2h, 3h) a defined aliquot was taken and plated on blood agar plates after serial dilution to determine the colony forming units (cfu)/mL. The rest of the bacterial solution of one well were transferred to a reaction tube. Bacterial cultures were spinned down, washed twice with PBS to remove unbound tracer and finally the counts associated with the bacterial pellet were determined using a gamma-counter (Wizard2 gamma counter, Perkin-Elmer Life Science). The decay corrected tracer radioactivity was normalized to bacterial cfu.

[40] M.A. Hackel, M. Tsuji, Y. Yamano, R. Echols, J.A. Karlowsky, and D.F. Sahm, *Diagn. Microbiol. Infect. Dis.*, 2019, **94**, 321-325.
[41] T. Bremell, A. Abdelnour, and A. Tarkowski, *Infect. Immun.*, 1992, **60**, 2976-2985.

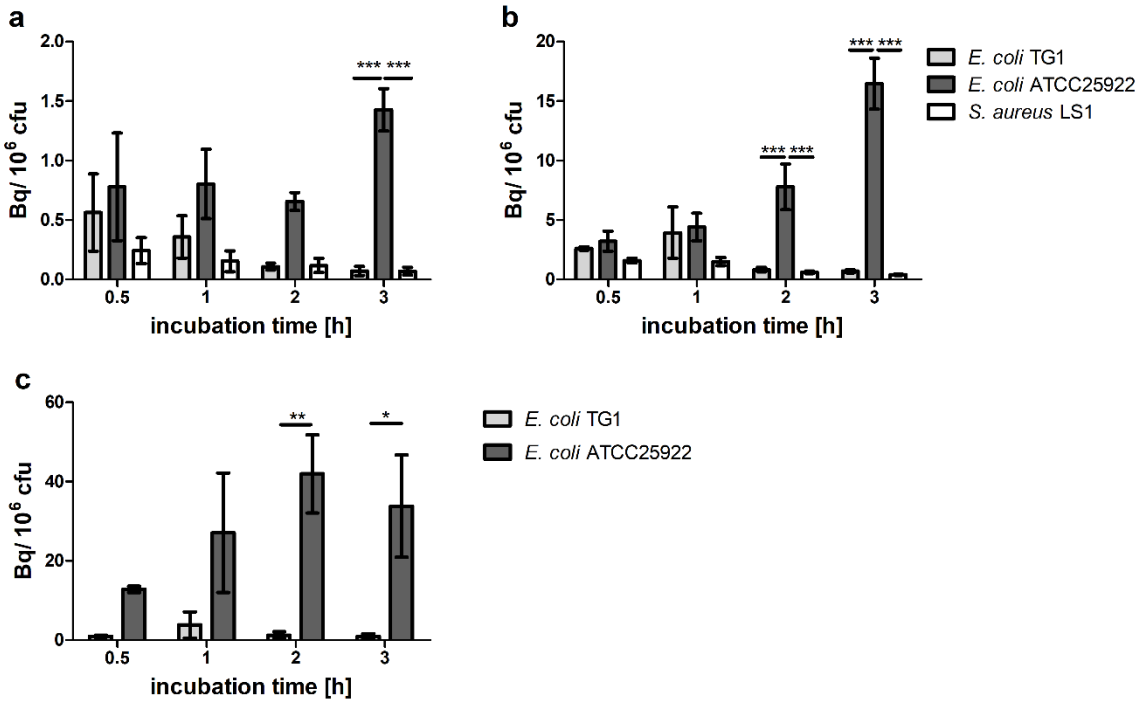
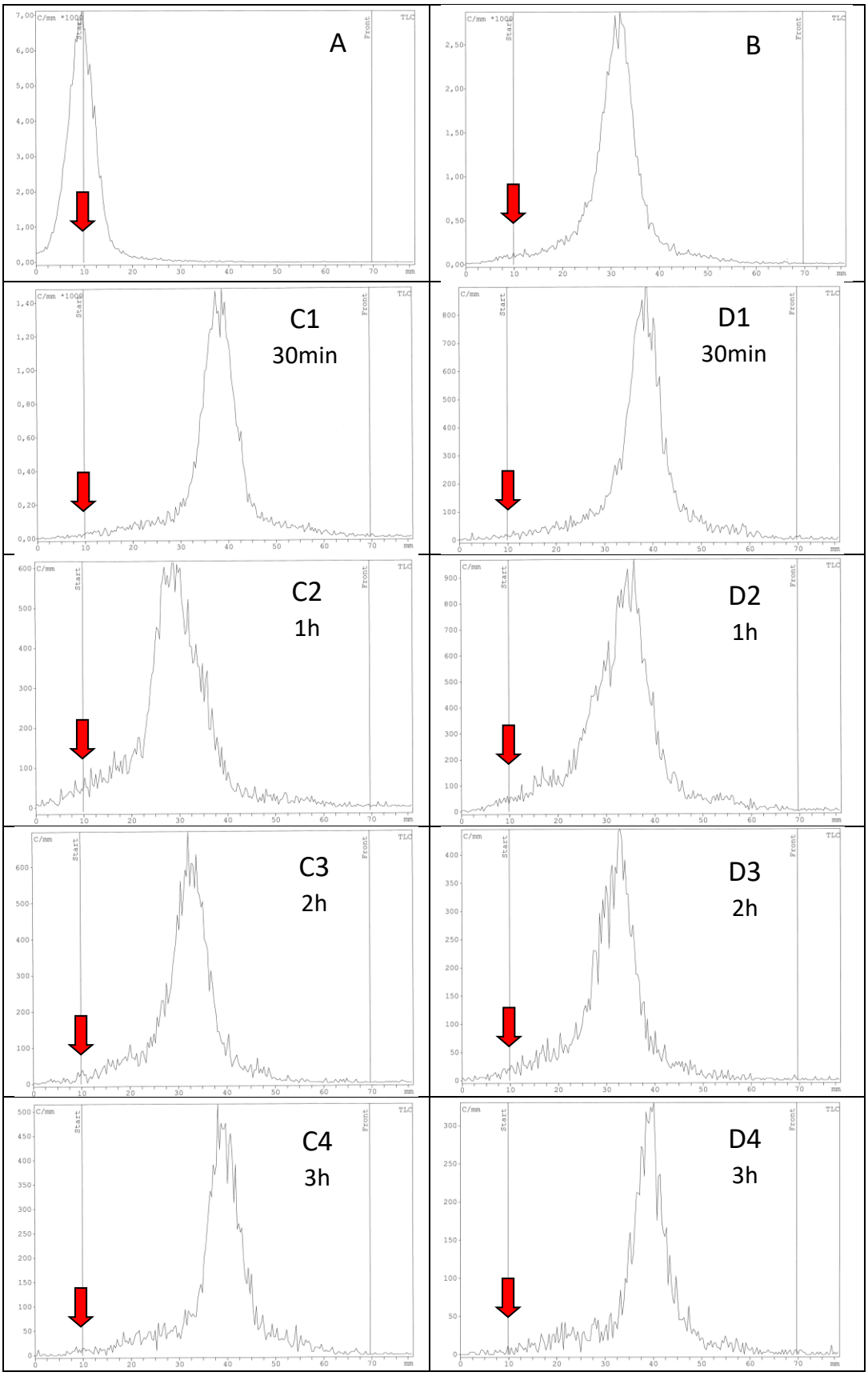


Fig.S3: Strain, tracer concentration and iron dependent *in vitro* uptake of **[^{68}Ga]Ga-RMA693**. (a) *Escherichia coli* TG1, *E. coli* ATCC25922 and *S. aureus* LS1 were incubated with 0.1 MBq/ml **[^{68}Ga]Ga-RMA693** in Mueller-Hinton-broth (MHB) for up to three hours. (b) Bacteria were incubated with 1 MBq/ml **[^{68}Ga]Ga-RMA693** in MHB (c) *E. coli* TG1, and *E. coli* ATCC25922 were incubated with 0.1 MBq/ml **[^{68}Ga]Ga-RMA693** in iron-depleted cation-adjusted MHB for up to three hours. Tracer uptake was determined using a gamma counter and bacterial colony forming units (cfu) by serial dilution and plating. Data represents the means ± SEM of three independent experiments. *, $p < 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; two-way ANOVA followed by Bonferroni posttests.

Complex stability test (MHB and ID-CAMHB):

The metal exchange stability test of [^{68}Ga]Ga-RMA693 in media used for *in vitro* experiments was performed by incubation of pure product (about 20 MBq) in both media (in iron-depleted cation-adjusted Mueller Hinton Broth (ID-CAMHB) and Mueller-Hinton-broth (MHB)) at 37°C up to 180 minutes (sampling times: 30min, 1h, 2h, 3h). Degradation of complex [^{68}Ga]Ga-RMA693 and releasing of free ^{68}Ga was evaluated by radio-TLC (Silica, ACN/H_2O 4:1). The TLC plates were after developing scanned on gamma scanner. Eventually released free colloidal ^{68}Ga would stay on start of TLC plate. In both media, no free ^{68}Ga-acticvity is detectable up to 3h of incubation. [^{68}Ga]Ga-RMA693 is stable in both media.

Fig. S4: Determination of stability in media (see next page). Chromatograms: (A) TLC of the free ^{68}Ga activity (prepared on the same way as the labeled compound **[^{68}Ga]Ga-RMA693**, but without adding the ligand); (B) TLC of **[^{68}Ga]Ga-RMA693**; (C1-4) stability of **[^{68}Ga]Ga-RMA693** in iron-depleted cation-adjusted Mueller Hinton Broth (ID-CAMHB); (D1-4) stability of **[^{68}Ga]Ga-RMA693** in Mueller-Hinton-broth (MHB); red arrow shows position of free ^{68}Ga activity.


A
B
C1
30min
D1
30min
C2
1h
D2
1h
C3
2h
D3
2h
C4
3h
D4
3h

5. *In vivo* experiments

Animal approval

All animal experiments were approved by the North Rhine-Westphalia Agency for Nature, Environment, and Consumer Protection (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen-LANUV; ID 81-02.04.2020.A402).

Mouse model of subcutaneous infection

In preparation for the mouse model bacteria (*E. coli* ATCC25922, or TG1) from overnight culture in MHB (shaking conditions, 37 °C) were adjusted to OD_{600} 1 in PBS with 15 % glycerol and stored in aliquots at −20 °C until use. From a previously frozen aliquot the number of cfu was determined after serial dilutions of the bacterial suspensions on blood agar and overnight incubation at 37 °C. For the experiment, a bacterial aliquot was thawed and washed twice with PBS to remove the glycerol. Afterwards, the bacteria were adjusted to $1x10^8$ bacteria/mL with PBS.

In this study female CD-1 mice with an age of 10-12 weeks were used. Mice were infected subcutaneously with *E. coli* ATCC25922 in the shoulder area with $5x10^6$ bacteria/ 50 µL. *E. coli* TG1 was implanted on the contralateral side.

PET/CT imaging

PET images were obtained using a quadHIDAC PET camera (Oxford Positron Systems Ltd., Oxford, UK). Three hours after infection, ~10MBq [^{68}Ga]Ga-RMA693 were injected intravenously into anesthetized mice (isoflurane in O_2) and images were acquired either dynamically 0 – 90 min after tracer injection (*n* = 2 healthy mice (biodistribution) and *n* = 2 infected mice) or statically (75 – 90 min after tracer injection, *n* = 4 healthy mice (biodistribution) and *n* = 4 infected mice). Subsequently, CT images were acquired with an Inveon CT scanner (Siemens). Using external markers, reconstructed PET images and CT images were coregistered to allow definition of specific volumes of interest for image quantification. Tracer accumulation was calculated as mean % injected dose (ID)/ mL.

After the PET/CT scan, tissues were harvested and radioactivity was measured on a gammacounter (Wizard2 gamma counter, Perkin-Elmer Life Science). Subsequently, the bacterial pellet was processed to determine the bacterial load. Therefore, the tissue was homogenized, serially diluted and plated on blood agar plates.

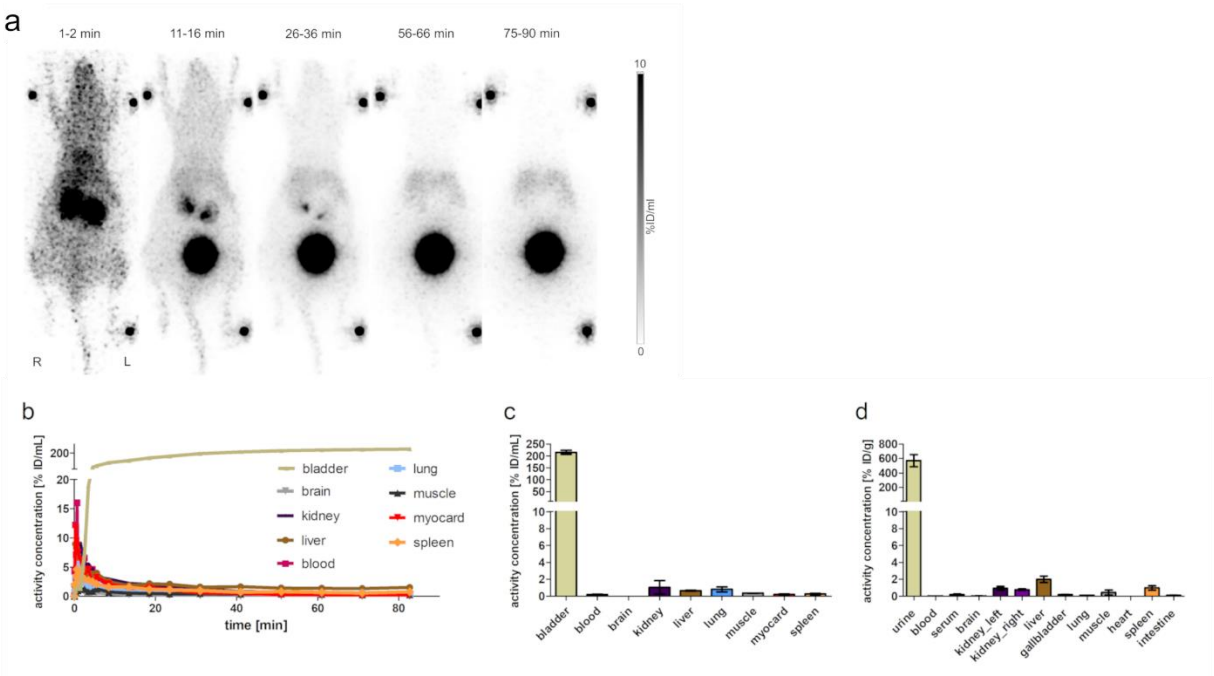


Fig.S5: *In vivo* distribution of **[^{68}Ga]Ga-RMA693** in a healthy mouse within 90 min after intravenous injection. (a) PET images (maximum intensity projections) reveal fast elimination of **[^{68}Ga]Ga-RMA693** from the blood through the kidneys into the urinary bladder, and did not show any accumulation of the radiotracer in tissues not involved in tracer elimination or excretion. (b) Quantitative analysis of a dynamically measured mouse confirmed the visual impression of fast blood clearance. (c) Quantitative analysis of the final imaging time frame (75 – 90 min post tracer injection) of a group of six mice; means ± SEM. (d) *Ex vivo* gamma counting of harvested organs and tissue of a group of six mice; means ± SEM. % ID = percentage of injected dose.

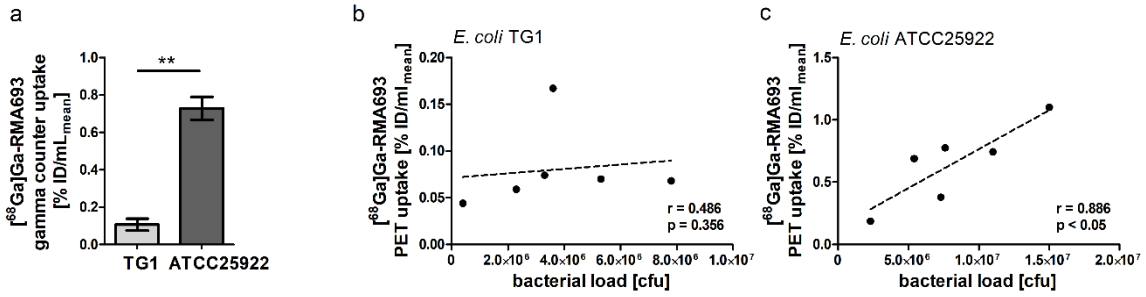


Fig.S6. (a) *Ex vivo* gamma counting of harvested tissue of a group of six infected mice; means ± SEM. (b, c) Spearman correlation between **[^{68}Ga]Ga-RMA693** PET uptake und viable bacteria in the tissue: (b) *E. coli* TG1 and (c) *E. coli* ATCC25922.

6. Statistical analysis

Statistical analyses of *in vitro* assays were performed with Prism (GraphPad Software) using using two-way ANOVA followed by Bonferroni post-test. $P < 0.05$ was considered statistically significant. The means from *in vivo* mouse models were compared using two-tailed Mann Whitney U test. Correlations between imaging signals and bacterial counts were performed using Spearman

correlation. The results are expressed as mean ± standard error of at least 3 independent experiments. $P < 0.05$ was considered statistically significant.

7. NMR and MS Spectra

7.1. 3-(Benzyloxy)-2-hydroxybenzaldehyde (5)

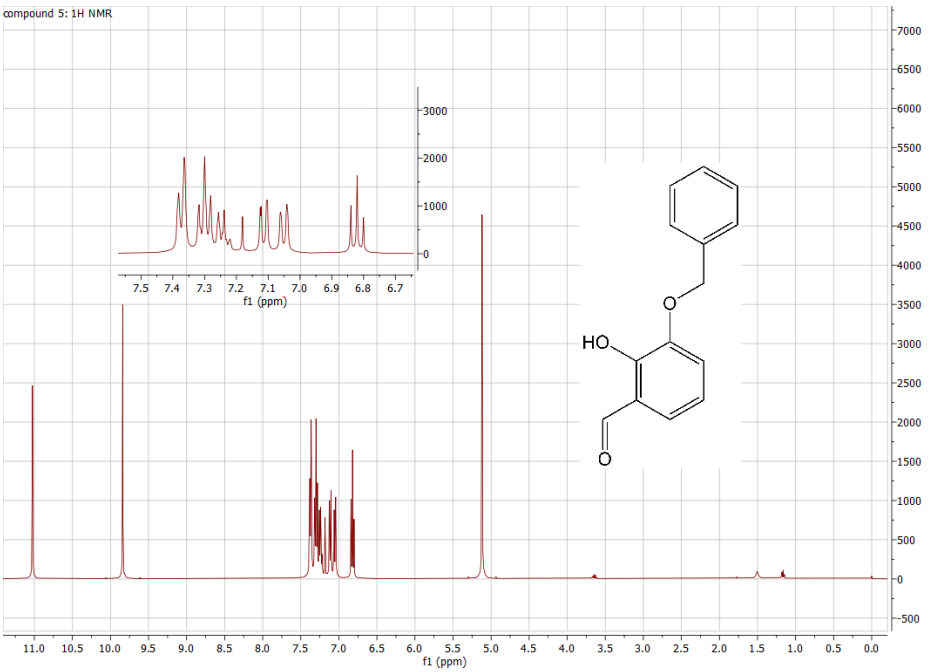


Fig.S7. ^{1}H-NMR spectrum (300MHz, $CDCl_3$) of 3-(Benzyloxy)-2-hydroxybenzaldehyde (**5**).

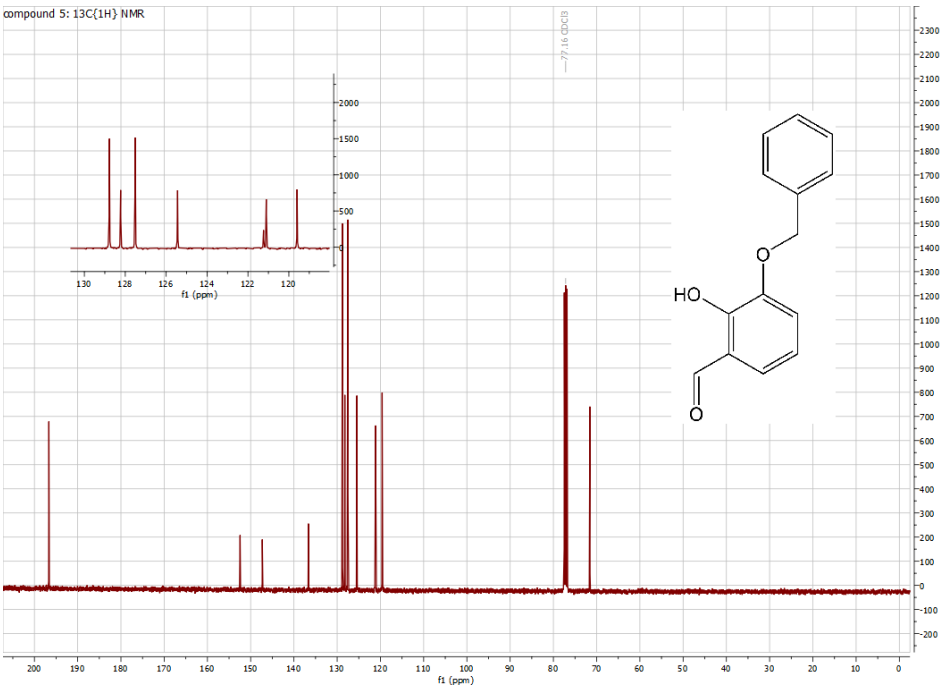


Fig.S8. $^{13}C\{^{1}H\}$-NMR spectrum (75MHz, $CDCl_3$) of 3-(Benzyloxy)-2-hydroxybenzaldehyde (**5**).

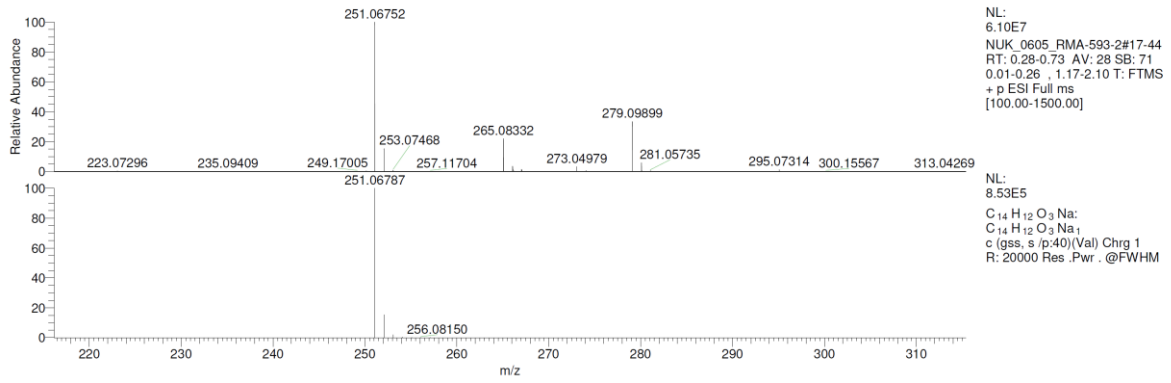


Fig.S9. Mass spectrum (HRMS (ESI+)) of 3-(Benzyloxy)-2-hydroxybenzaldehyde (**5**).

7.2. 3-(Benzyloxy)-5-iodo-2-hydroxybenzaldehyde (6)

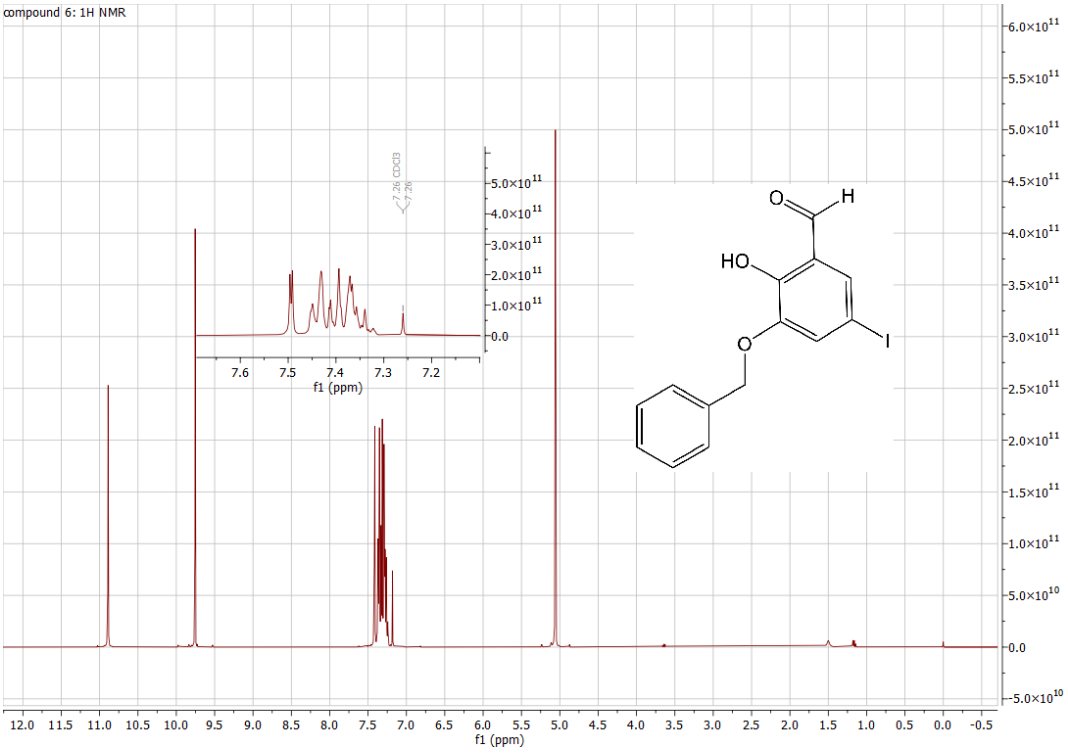


Fig.S10. ^{1}H-NMR spectrum (300MHz, $CDCl_3$) of 3-(Benzyloxy)-5-iodo-2-hydroxybenzaldehyde (**6**).

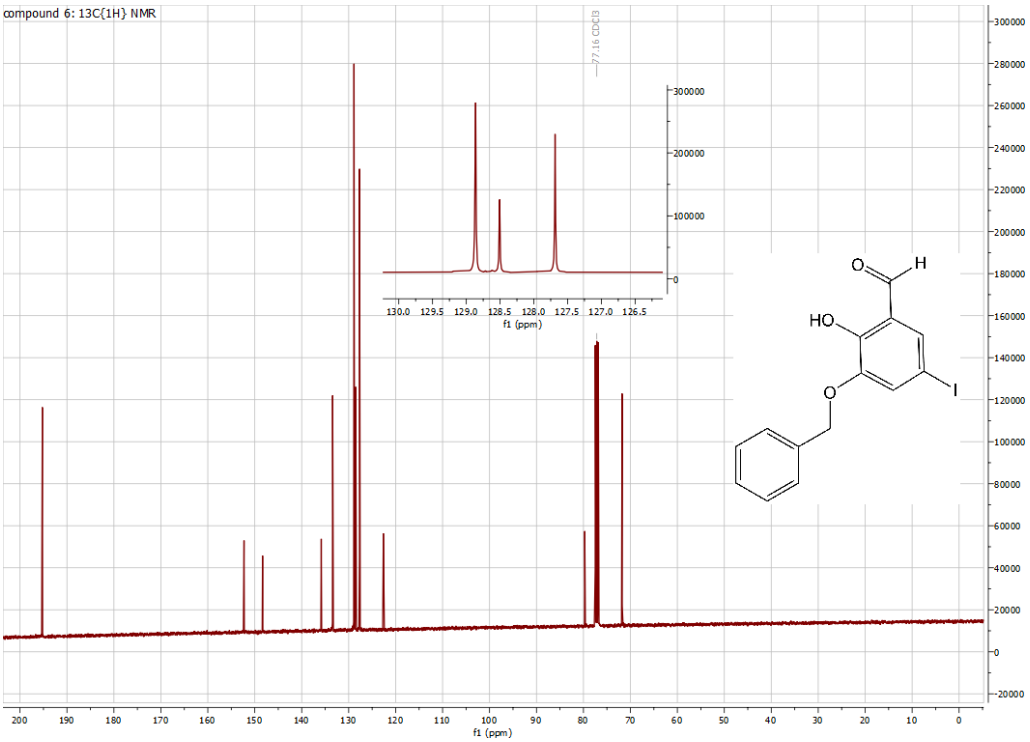


Fig.S11. $^{13}C\{^{1}H\}$-NMR spectrum (75MHz, $CDCl_3$) of 3-(Benzyloxy)-5-iodo-2-hydroxybenzaldehyde (**6**).

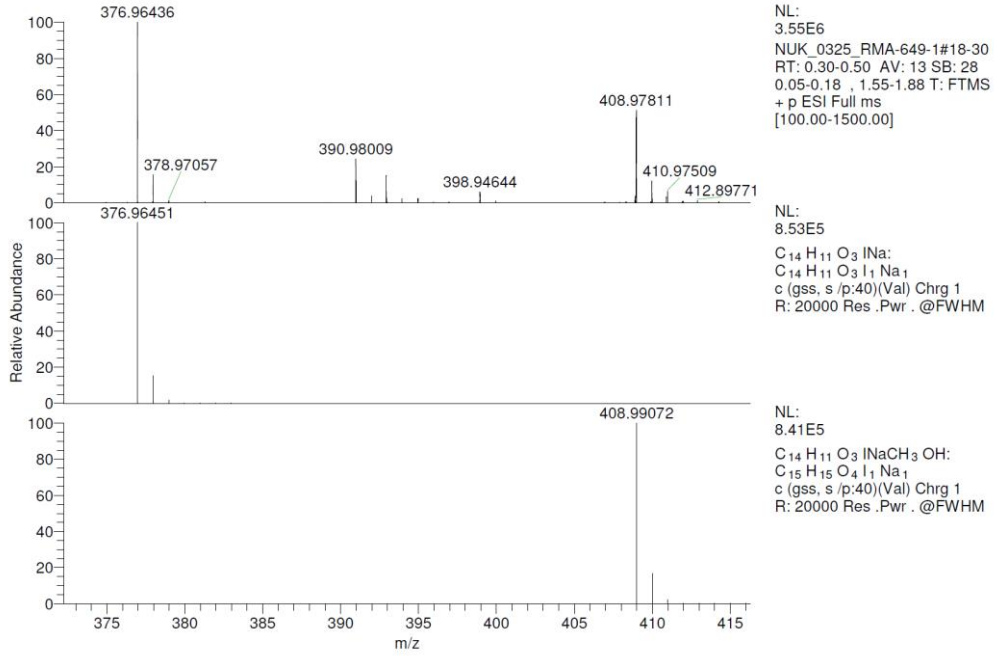


Fig.S12. Mass spectrum (HRMS (ESI+)) of 3-(Benzyloxy)-5-iodo-2-hydroxybenzaldehyde (**6**).

7.3. 2,3-Bis(benzyloxy)-5-iodo-benzaldehyde (**7**)

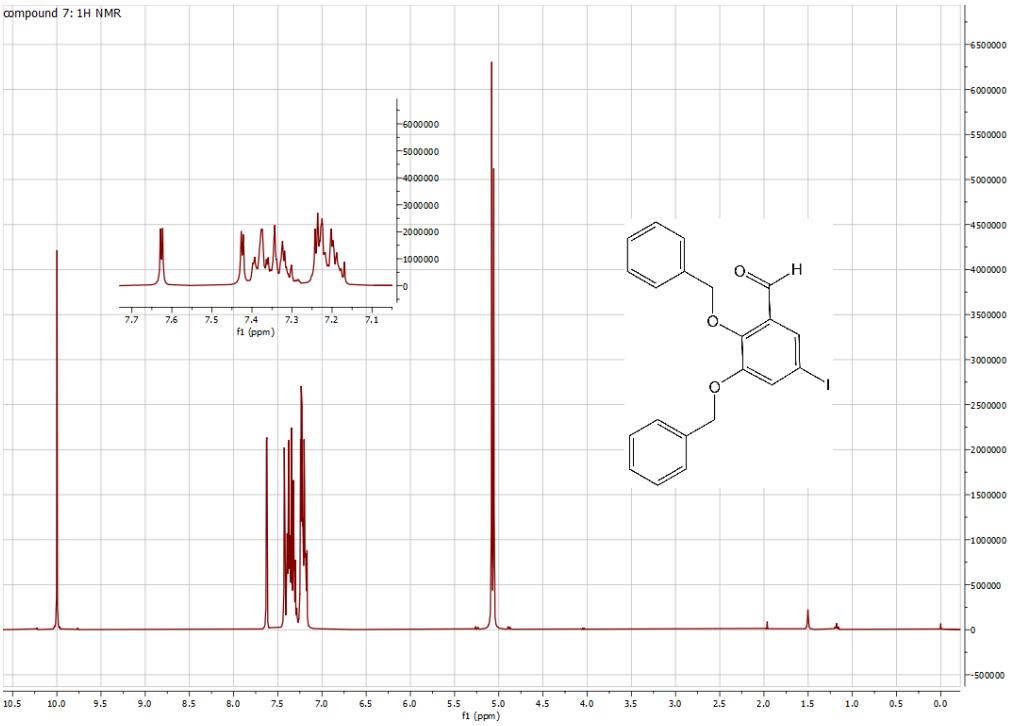


Fig.S13. ^{1}H-NMR spectrum (400MHz, $CDCl_3$) of 2,3-Bis(benzyloxy)-5-iodo-benzaldehyde (**7**).

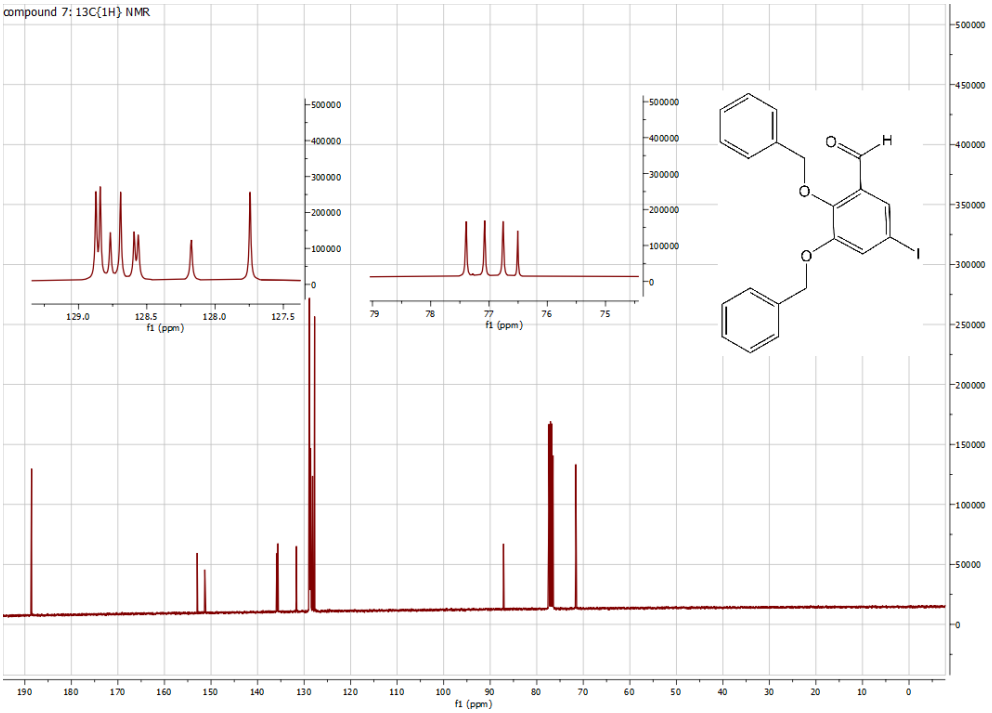


Fig.S14. $^{13}C\{^{1}H\}$-NMR spectrum (100MHz, $CDCl_3$) of 2,3-Bis(benzyloxy)-5-iodo-benzaldehyde (**7**).

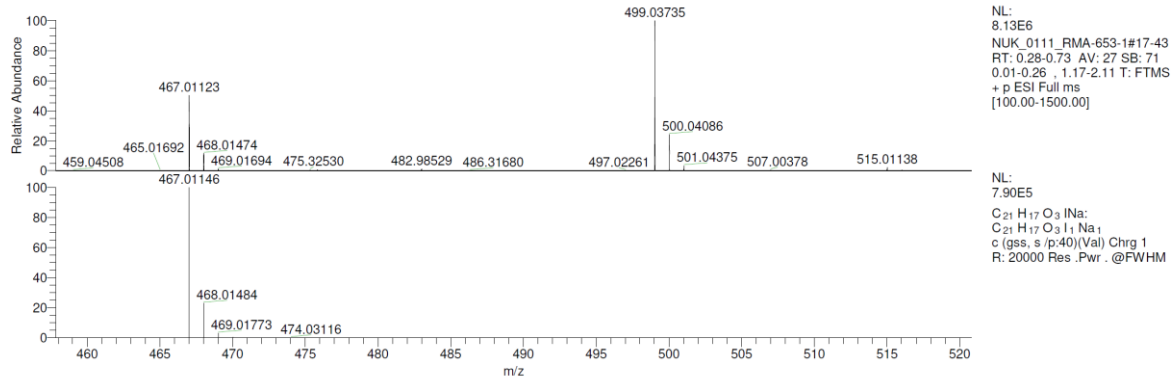


Fig.S15. Mass spectrum (HRMS(ESI+)) of 2,3-Bis(benzyloxy)-5-iodo-benzaldehyde (**7**).

7.4. (2,3-bis(benzyloxy)-5-iodophenyl)methanol (**8**)

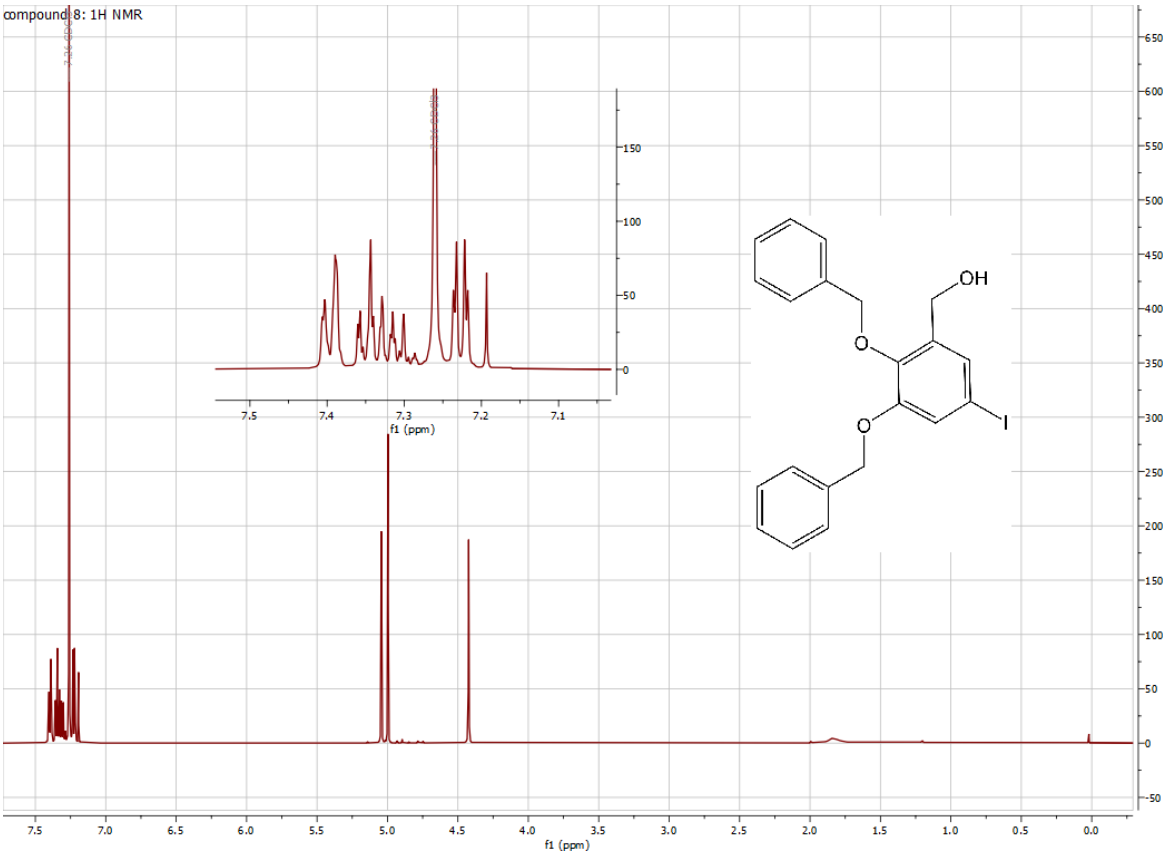


Fig.S16. ^{1}H-NMR spectrum (600MHz, $CDCl_3$) of (2,3-bis(benzyloxy)-5-iodophenyl)methanol (**8**).

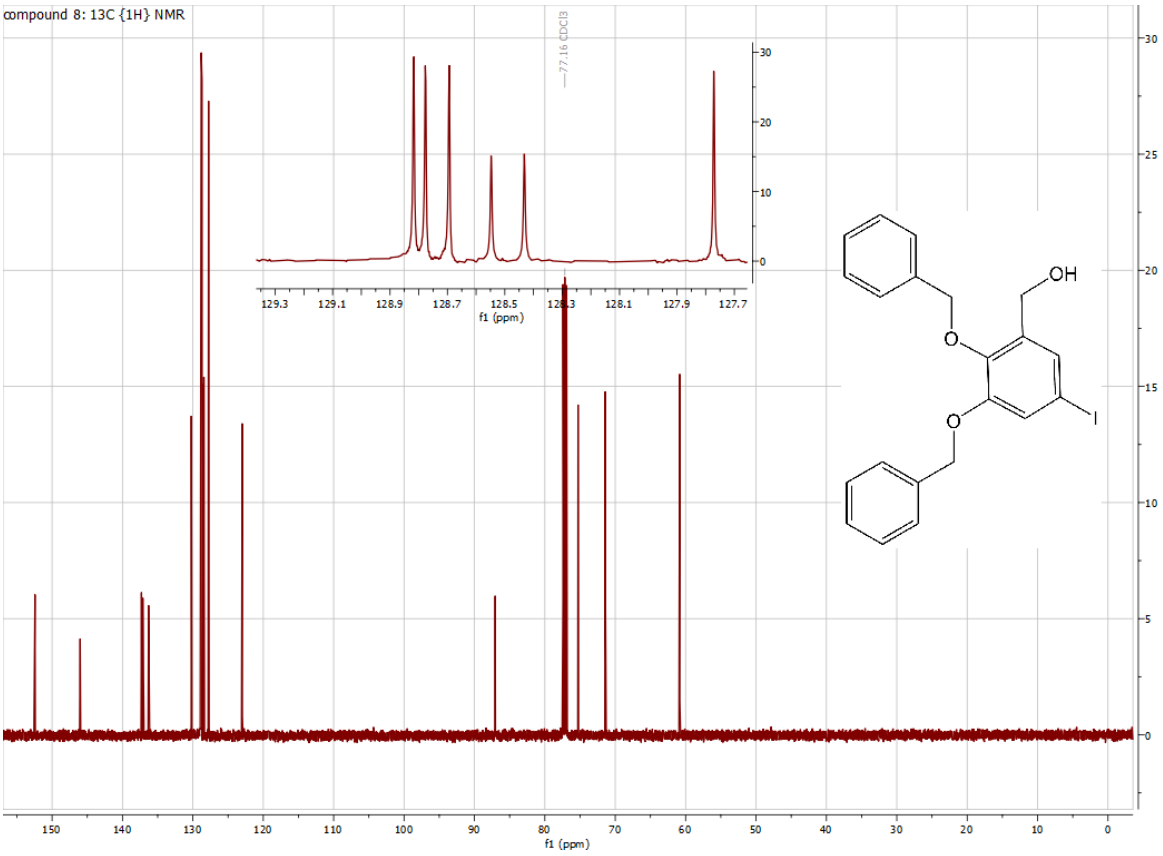


Fig.S17. $^{13}C\{^{1}H\}$-NMR spectrum (151MHz, $CDCl_3$) of (2,3-bis(benzyloxy)-5-iodophenyl)methanol (**8**).

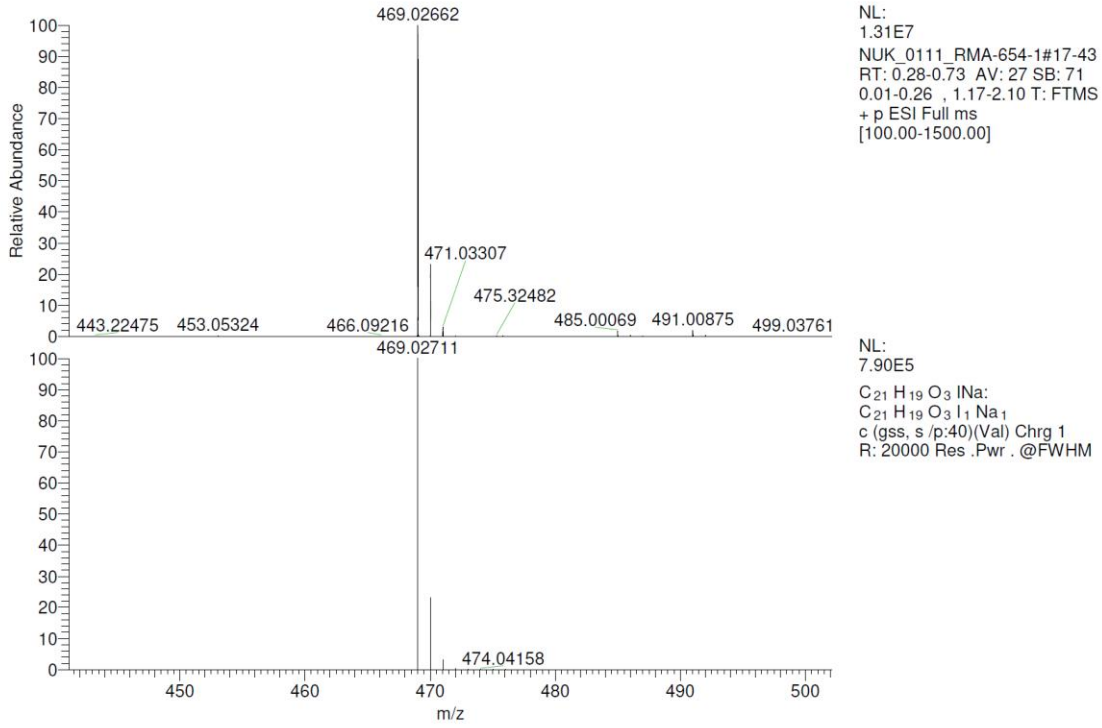


Fig.S18. Mass spectrum (HRMS(ESI+)) of (2,3-bis(benzyloxy)-5-iodophenyl)methanol (**8**).

7.5. ((2,3-bis(benzyloxy)-5-iodobenzyl)oxy)triisopropylsilane (**9**)

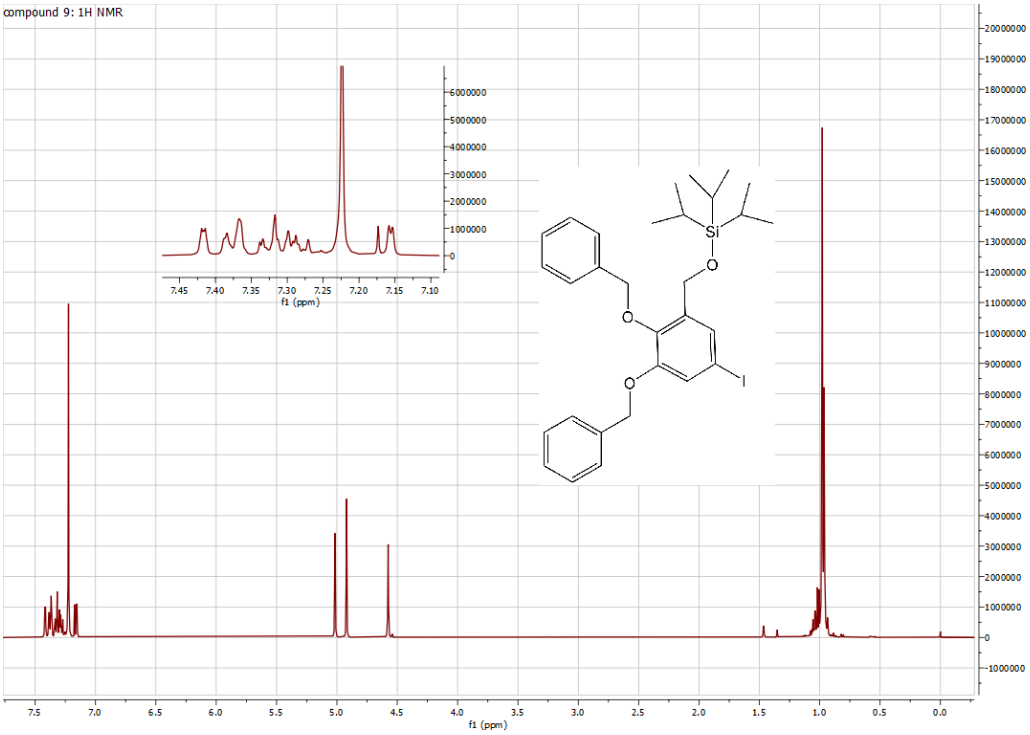


Fig.S19. ^{1}H-NMR spectrum (400MHz, $CDCl_3$) of ((2,3-bis(benzyloxy)-5-iodobenzyl)oxy)triisopropylsilane (**9**).

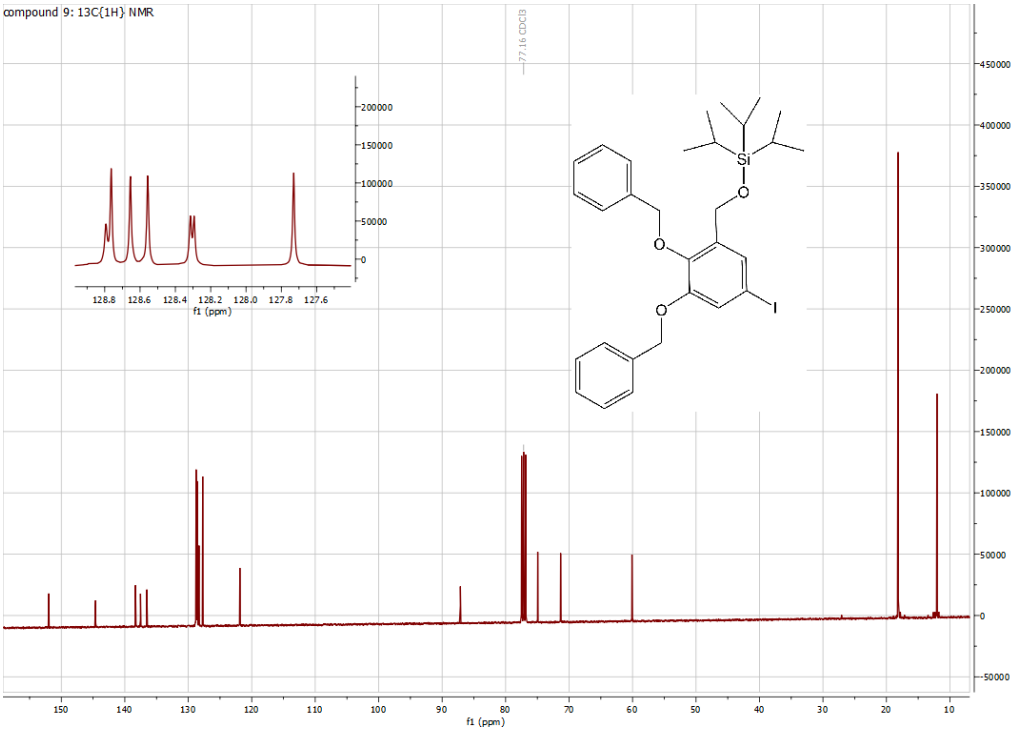


Fig.S20. $^{13}C\{^{1}H\}$-NMR spectrum (100MHz, $CDCl_3$) of ((2,3-bis(benzyloxy)-5-iodobenzyl)oxy)triisopropylsilane (**9**).

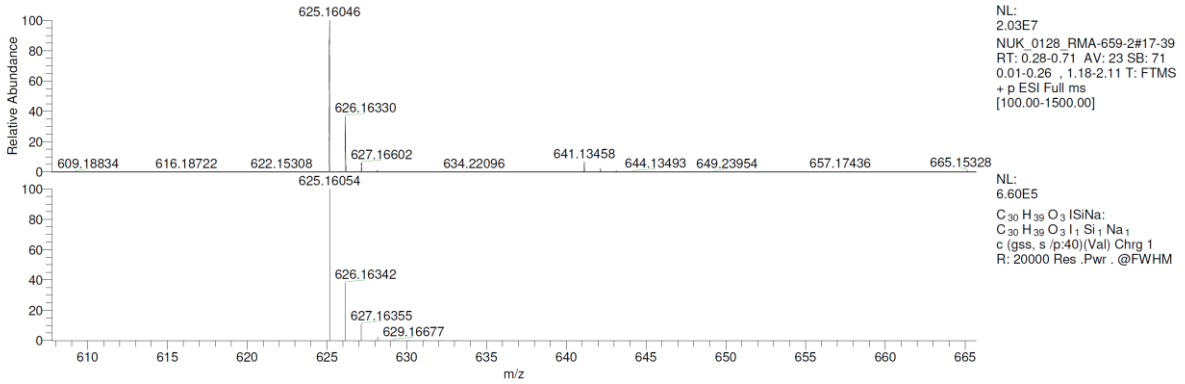


Fig.S21. Mass spectrum (HRMS(ESI+)) of ((2,3-bis(benzyloxy)-5-iodobenzyl)oxy)triisopropylsilane (**9**).

7.6. (3*R*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-2-(3,4-bis(benzyloxy)-5-(((triisopropylsilyl)oxy)methyl)phenyl)tetrahydro-2H-pyran-2-ol (**10**)

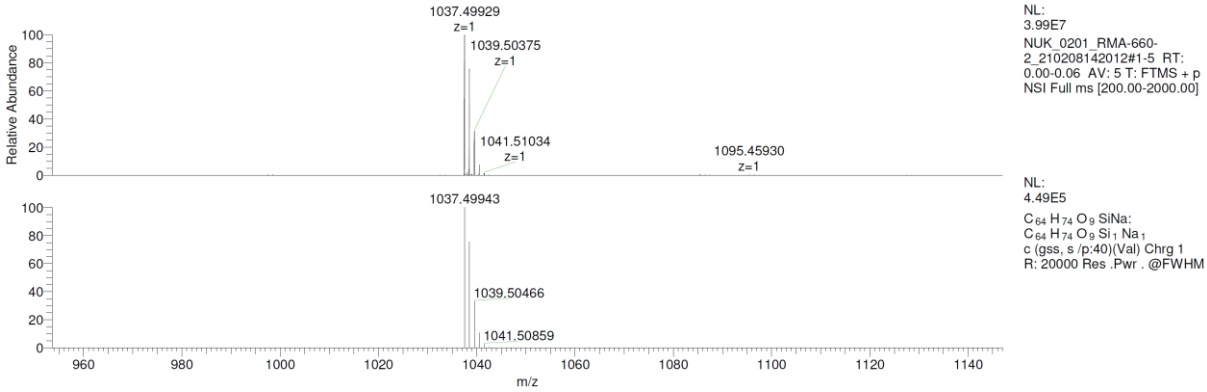


Fig.S22. Mass spectrum (HRMS(ESI+)) of **10**.

7.7. ((2,3-bis(benzyloxy)-5-((2*S*,3*S*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-tetrahydro-2H-pyran-2-yl)benzyl)oxy)triisopropylsilane (**11**)

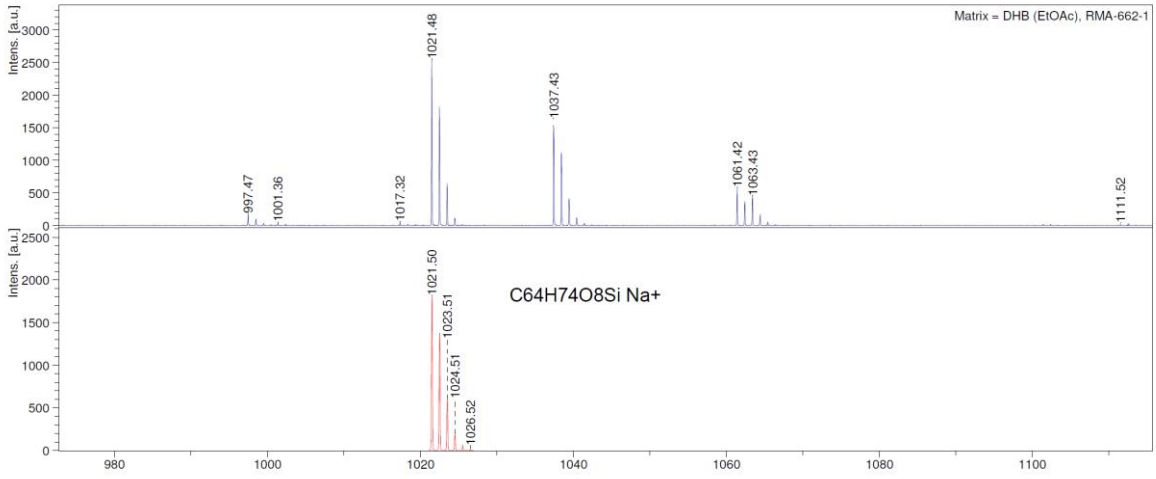


Fig.S23. Mass spectrum (MALDI-ToF MS (DHB, EtOAc)) of **11**.

7.8. (2,3-bis(benzyloxy)-5-((2*S*,3*S*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-tetrahydro-2H-pyran-2-yl)phenyl)methanol (**12**)

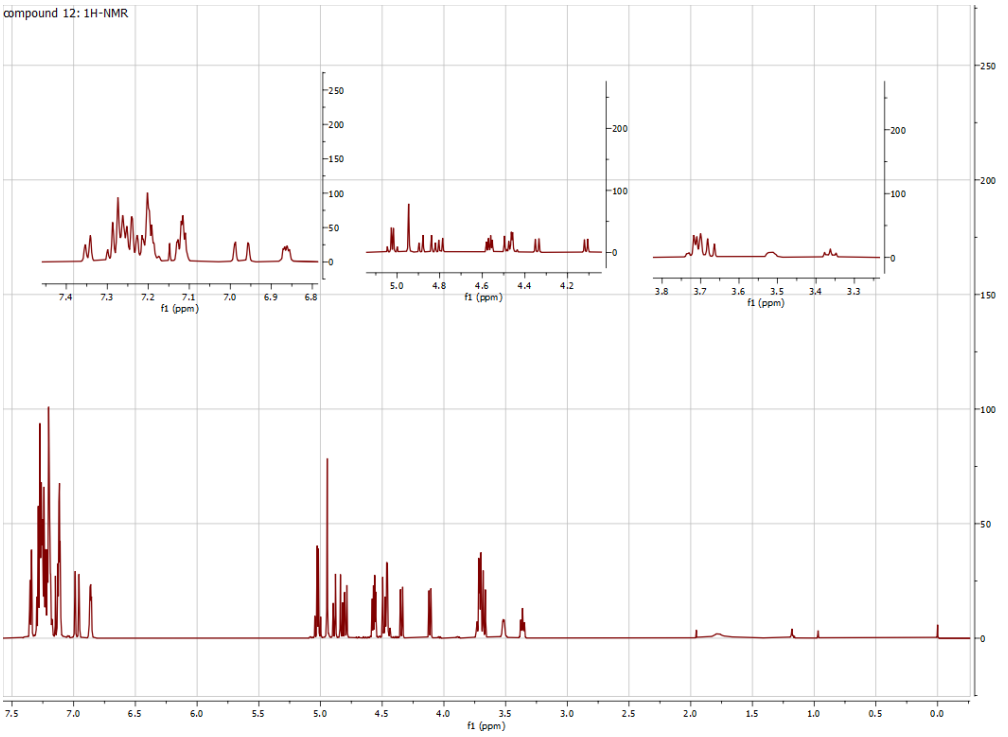


Fig.S24. ^{1}H NMR (600MHz, $CDCl_3$) of **12**.

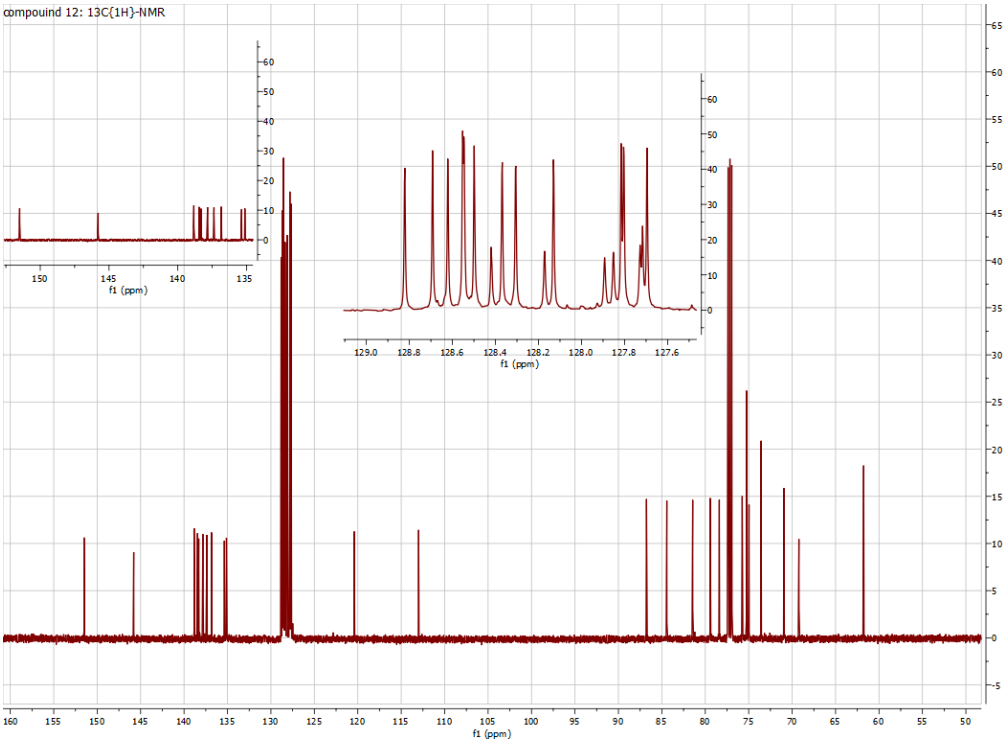


Fig.S25. $^{13}C\{^{1}H\}$ NMR (150MHz, $CDCl_3$) of **12**.

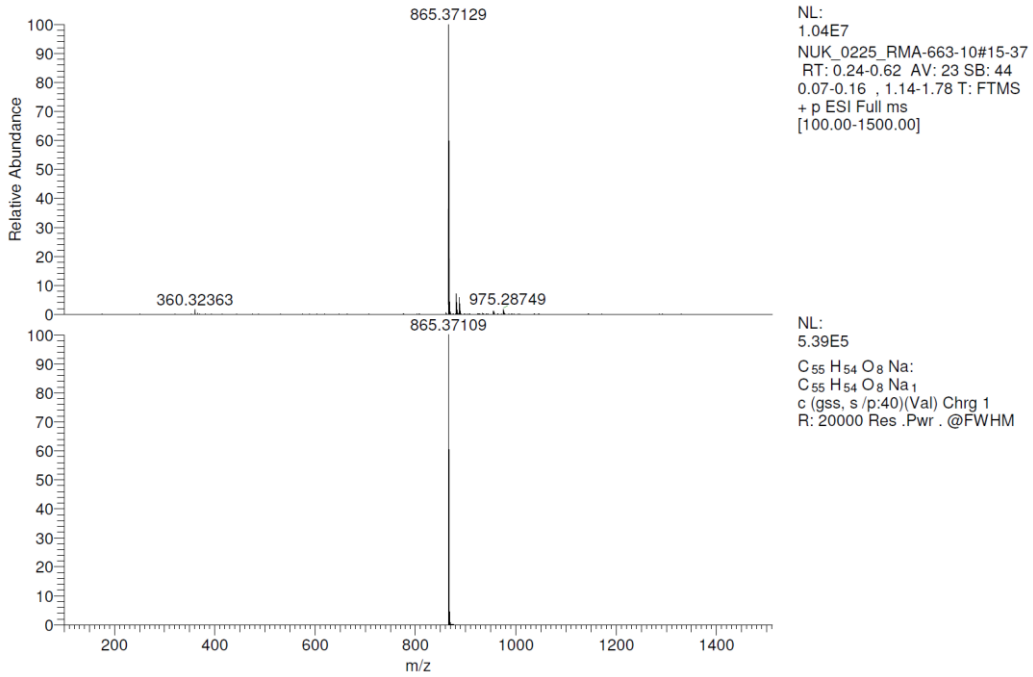


Fig.S26. Mass spectrum (HRMS(ESI+)) of **12**.

7.9. 2,3-bis(benzyloxy)-5-((2*S*,3*S*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-tetrahydro-2H-pyran-2-yl)benzaldehyde (**13**)

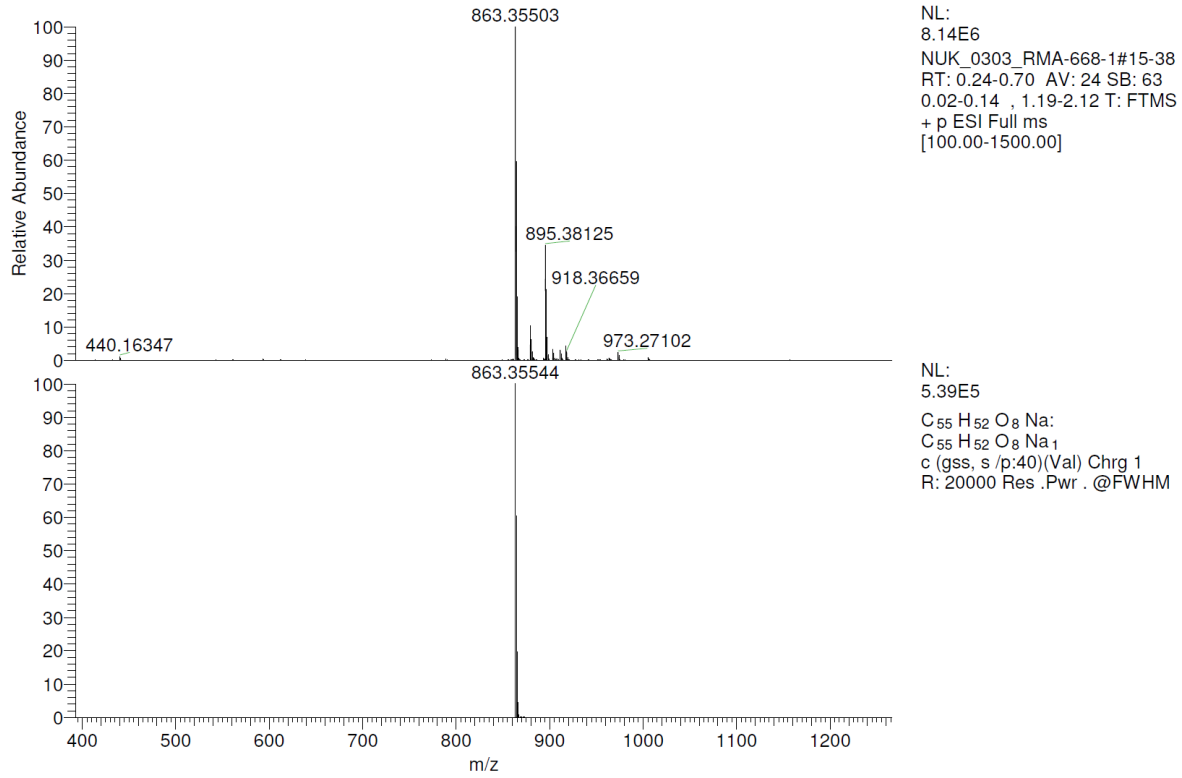


Fig.S27. Mass spectrum (HRMS(ESI+)) of **13**.

7.10. 2,3-bis(benzyloxy)-5-((2*S*,3*S*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-tetrahydro-2H-pyran-2-yl)benzoic acid (**14**)

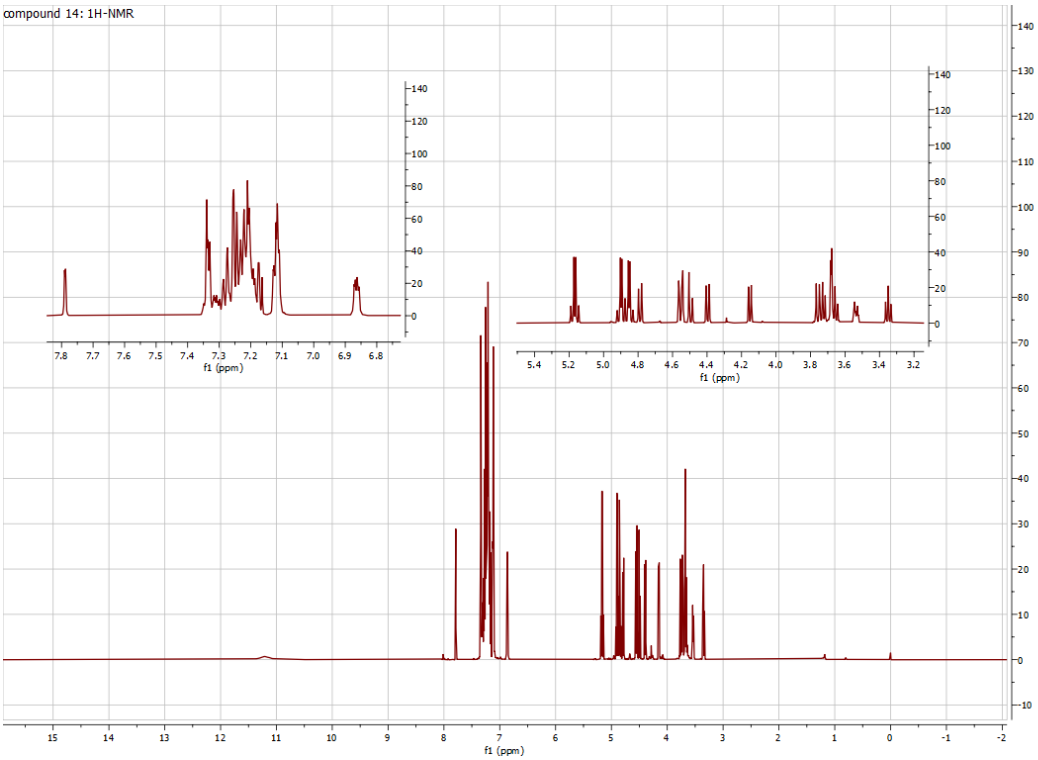


Fig.S28. ^{1}H NMR (600MHz, $CDCl_3$) of **14**.

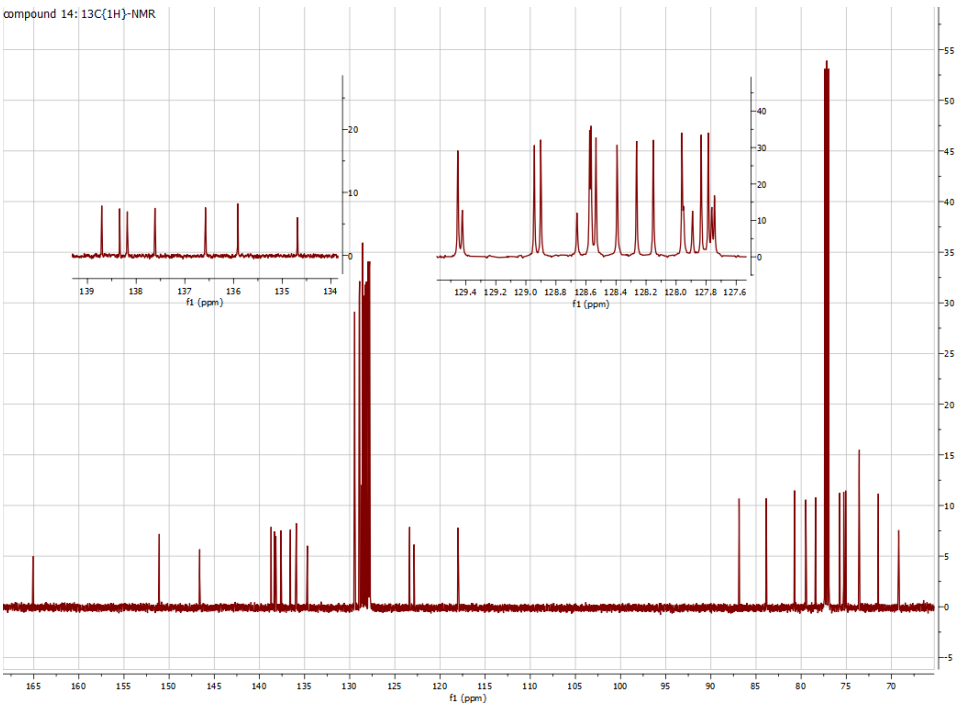


Fig.S29. $^{13}C\{^{1}H\}$ NMR (150MHz, $CDCl_3$) of **14**.

.

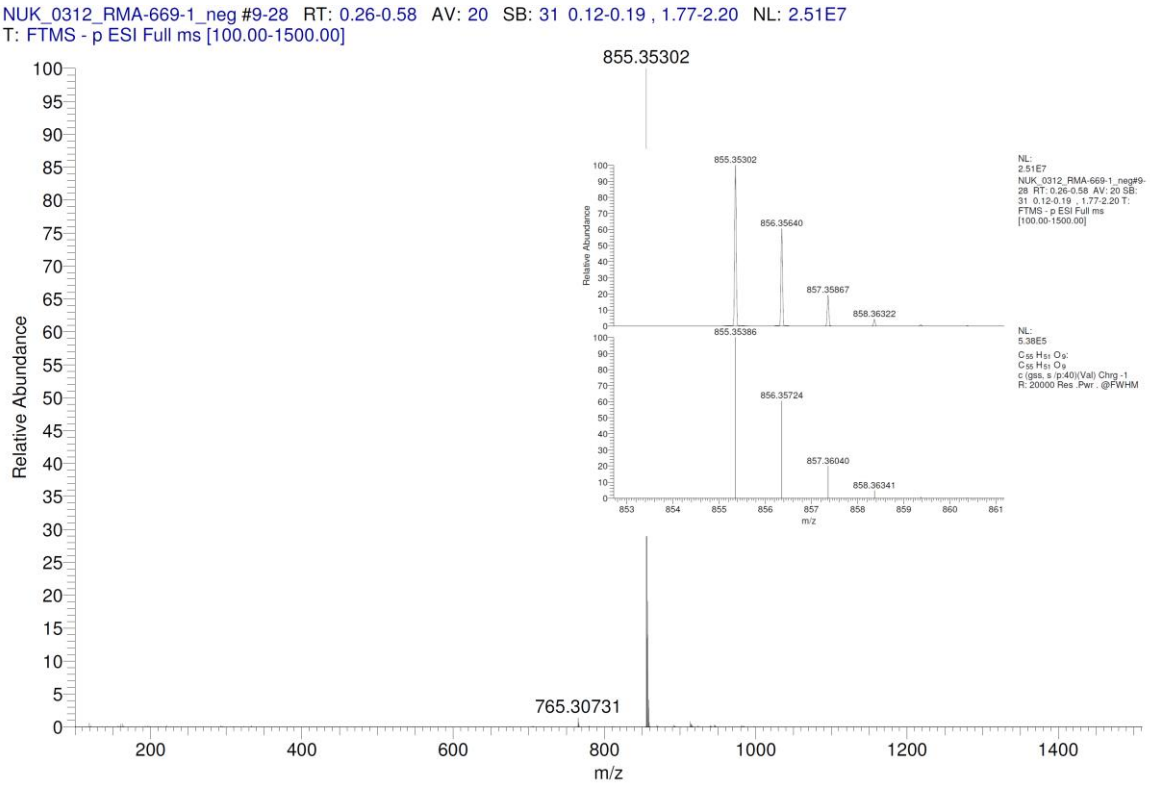


Fig.S30. Mass spectrum (HRMS(ESI-)) of **14**.

7.11. Perbenzylated RMA-693 (**15**)

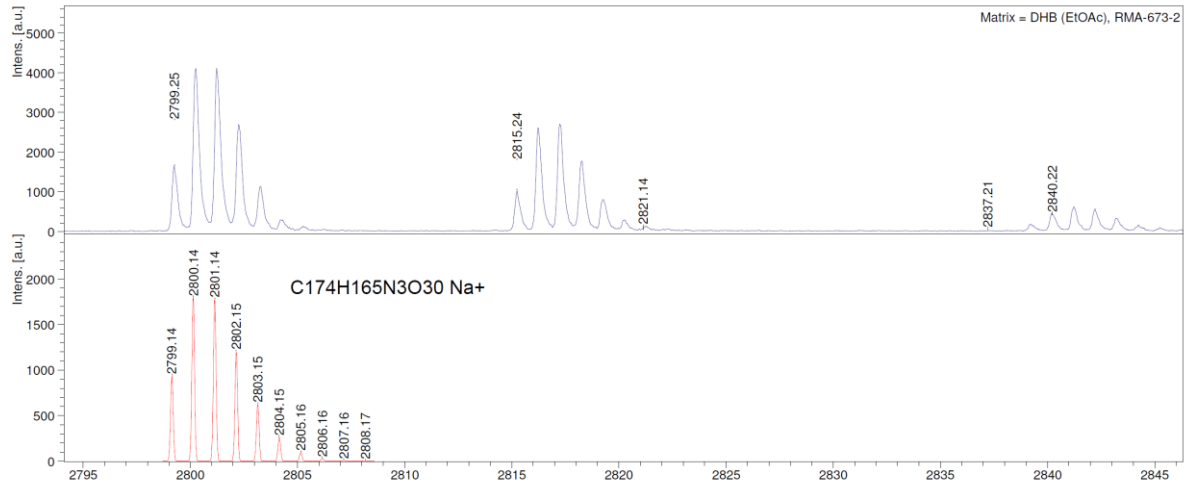


Fig.S31. Mass spectrum (MS-MALDI (DHB, EtOAc)) of **15**.

7.12. RMA693

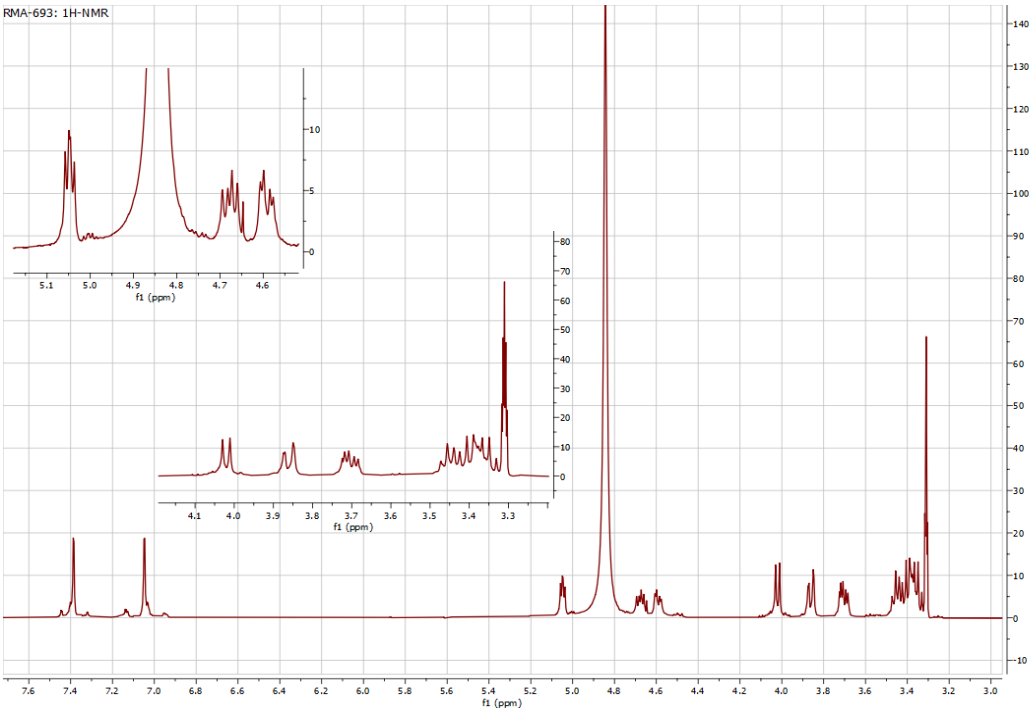


Fig.S32. ^{1}H NMR (500MHz, CD_3OD) of **RMA693**.

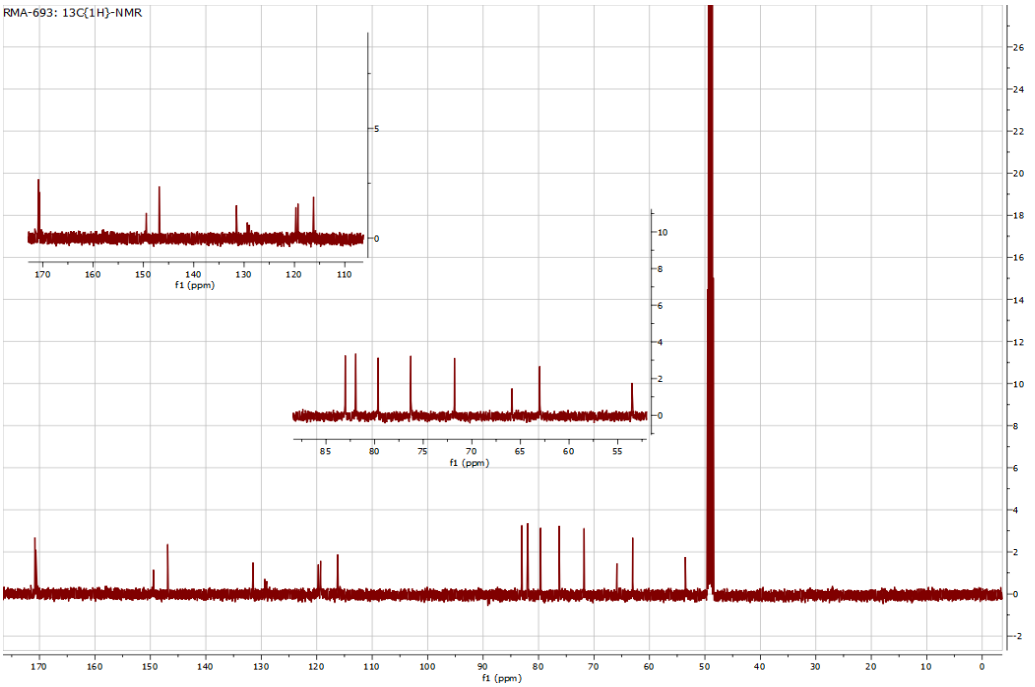


Fig.S33. $^{13}C\{^{1}H\}$ NMR (125MHz, CD_3OD) of **RMA693**.

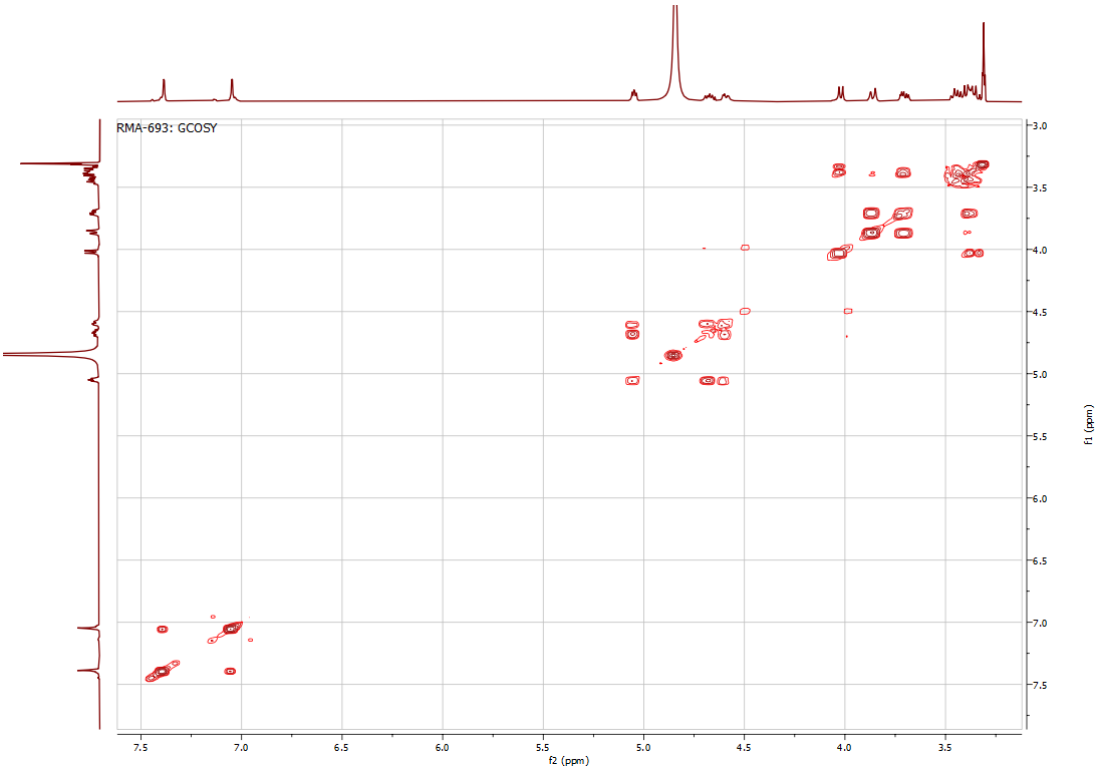


Fig.S34. NMR (GCOSY) of **RMA693**.

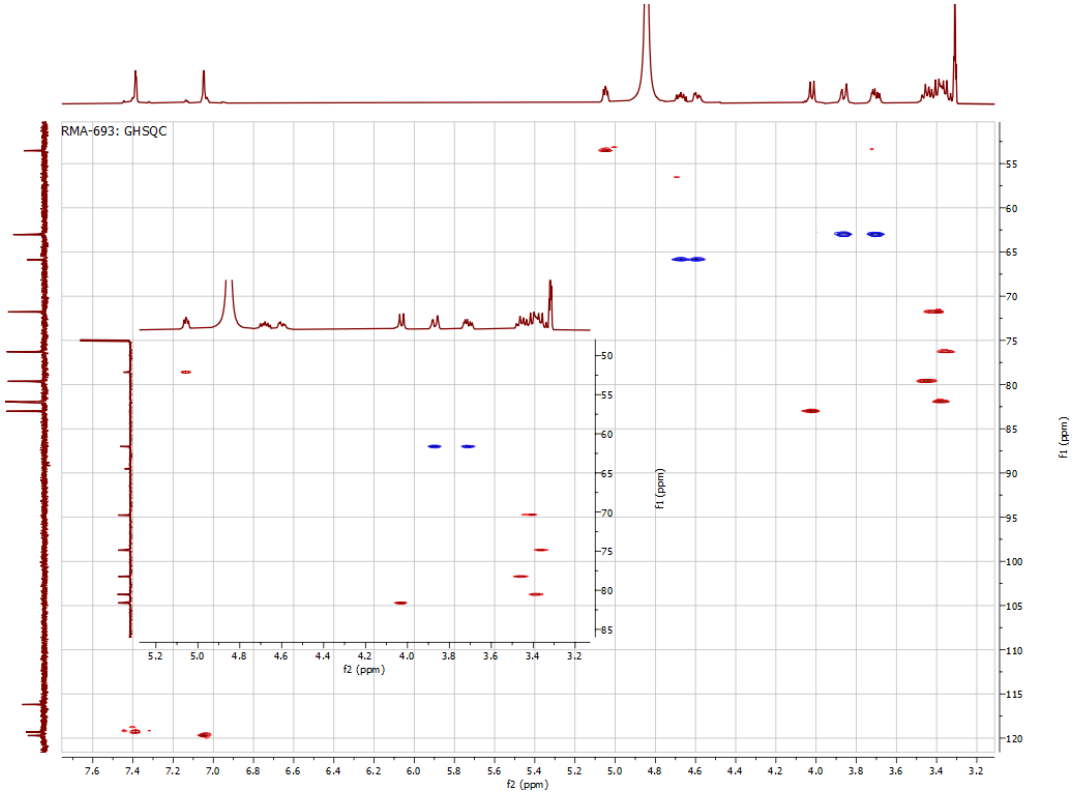


Fig.S35. NMR (GHSQC) of **RMA693**.

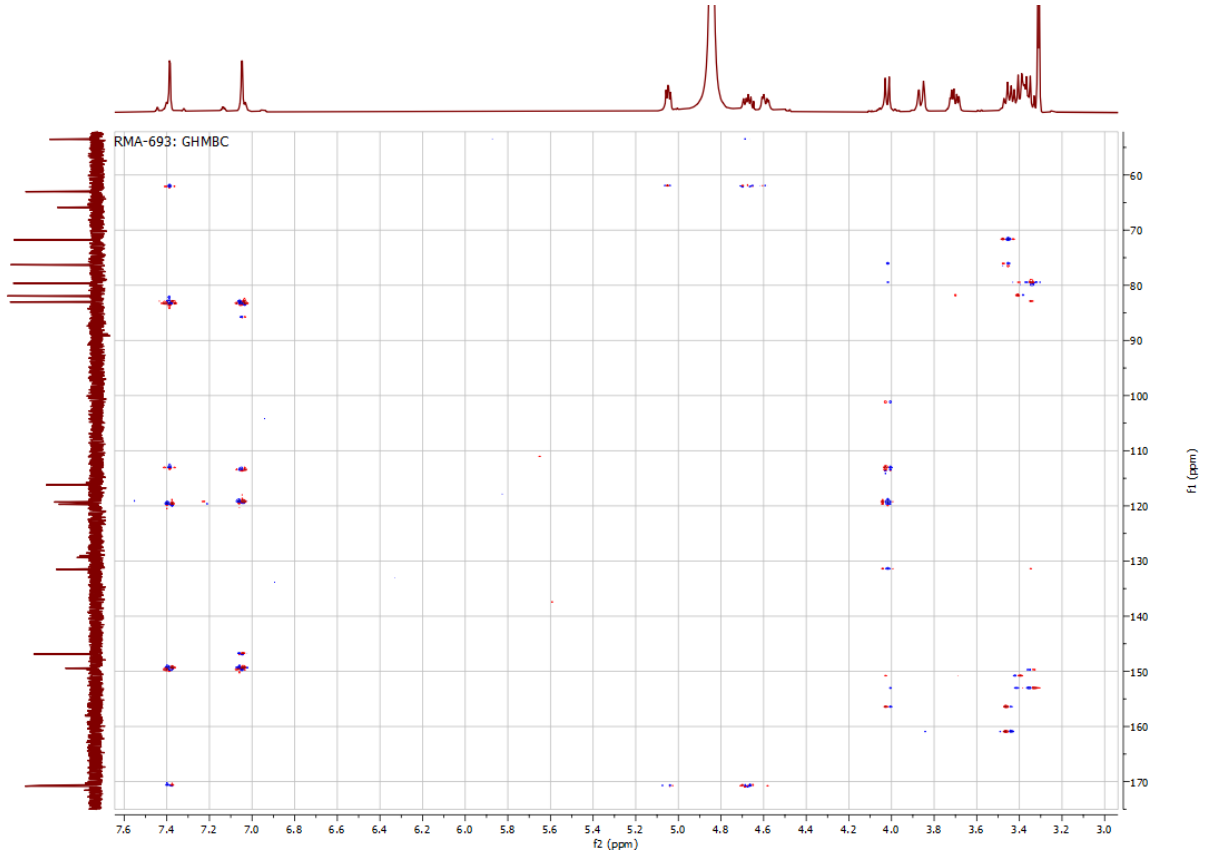


Fig.S36. NMR (GHMBC) of **RMA693**.

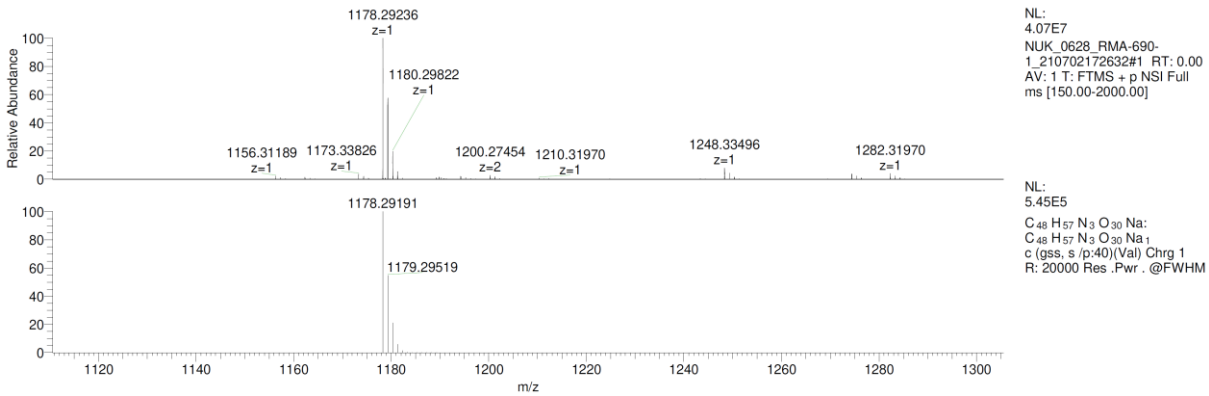


Fig.S37. Mass spectrum (HRMS(ESI+)) of **RMA693**.

7.13. Fe-RMA693 and Ga-RMA693

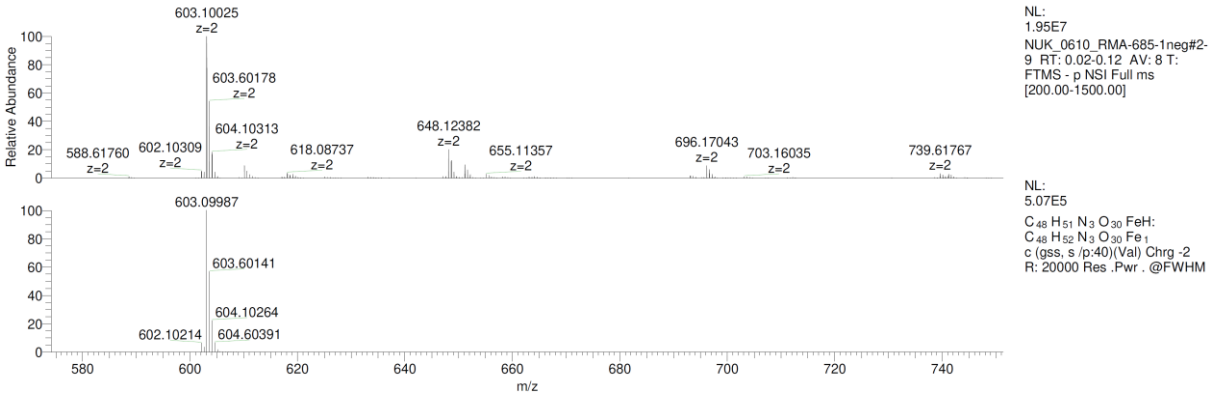


Fig.S38. Mass spectrum (HRMS(ESI+)) of **Fe-RMA693**.

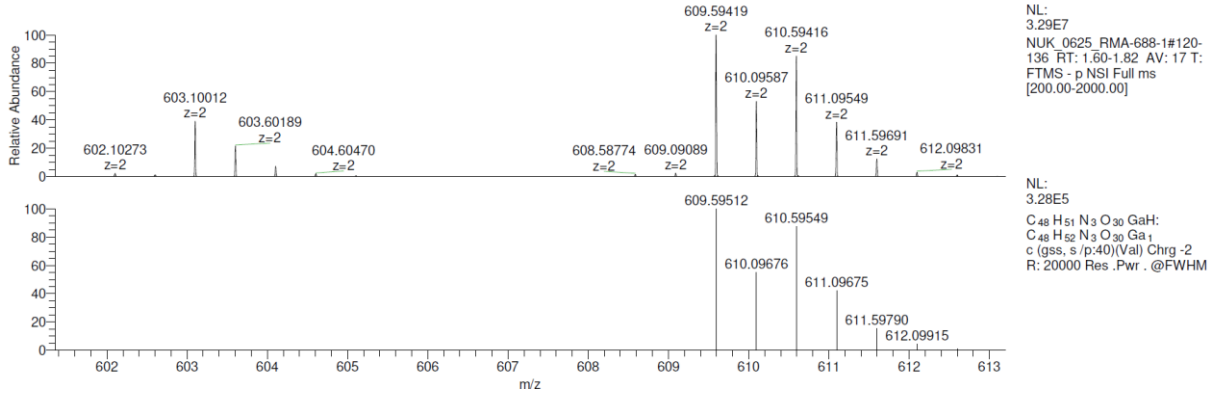


Fig.S39. Mass spectrum (HRMS(ESI+)) of **Ga-RMA693**.

8. Abbreviations

abs. = absolute

ACN = Acetonitrile

AcOH = Acetic acid

anhydr. = anhydrous

aq. = aqueous

Bn = Benzyl

cfu = colony forming units

conc. = concentrate

cyclohex. = cyclohexane

DCM = Dichloromethane (Methylene chloride)

decomp. = decomposition

det. = detection

DHB = 2,5-Dihydroxybenzoic acid (CAS: 490-79-9); matrix compound for MALDI-ToF MS

DIPEA = *N,N*-Diisopropylethylamine (Hünig's base)

DMP = Dess–Martin periodinane (CAS: 87413-09-0)

DMSO = Dimethyl sulfoxide

eq. = equivalent

ESI = Electrospray ionization

EtOAc = Ethyl acetate

EtOH = Ethanol HILIC = hydrophilic interaction liquid chromatography

g. = gas

glac. = glacial

HPLC = High-performance liquid chromatography

HRMS = High-resolution mass spectrometry

ID = injected dose

ID-CAMHB = iron-depleted cation-adjusted Mueller Hinton Broth

MALDI-ToF MS = Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry

MeOH = Methanol

MHB = Mueller Hinton Broth

mp = melting point

n-Bu = n-Butyl

NMR = Nuclear magnetic resonance

NSI = Nanospray ionisation

PBS = Phosphate buffered saline; isotonic buffer solution (pH = 7.4) commonly used in biological research

PTFE = Polytetrafluoroethylene, Teflon™

Py = Pyridine

PyAOP = 7-Azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (CAS: 156311-83-0); coupling reagent for peptide synthesis

R_f = Retention factor

Rt = Retention time

rt = room temperature

sat. = saturated

sol. = solution

SPE = Solid Phase Extraction

TIPSCl = Triisopropylsilyl chloride; Triisopropylchlorosilane (CAS: 13154-24-0)

TLC = Thin Layer Chromatography

TMHD = 2,2,6,6-Tetramethyl-3,5-heptanedione; dipivaloylmethane (CAS: 1118-71-4)

TMS = tetramethylsilane

UV = Ultraviolet

UV/Vis = Ultraviolet-visible

v/v = volume ratio