The first 2^{IB},3^{IA}-heterodifunctionalized β-cyclodextrin derivatives as an artificial enzyme

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Supporting Information available

Contents

1. General

Synthesis were monitored by thin-layer chromatography (TLC) on a plate of silica gel 60 F254 (E. Merck, Darmstadt, Germany). Developed plates were visualized using ultraviolet light and/or by dipping the plates into a solution of sulfuric acid in ethanol (8%) followed by heating with a heat gun. Column chromatographies were performed on silica gel 60 (0.04–0.063 mm, E. Merck). Flash chromatographies were performed on an automated apparatus Reveleris® (Grace Davison Discovery Sciences).

Methyl 5-(bromomethyl)-2-iodobenzoate and 4-chloromethyl-1-(triphenylmethyl)-imidazole used for the substitution of cyclodextrin derivatives were prepared as previously described.[1]

A detailed structural analysis is explained for compound **3** as a specific and representative example. HRMS and MS/MS analyses in the positive ion mode were performed on a Q-TOF Synapt G2-Si (Waters, Manchester, UK), equipped with an electrospray ionization (ESI) source (Z-spray) and an additional sprayer for the reference compound (Lock Spray). The compound **3** was dissolved in methanol, and the solution was directly introduced (5µL/min) through an integrated syringe pump into the electrospray source. The source and desolvation temperatures were 120 and 300 °C, respectively. Nitrogen was used as the drying and nebulizing gas at flow rates of 500 and 50 L/h, respectively. The capillary voltage was 2.5 kV and the cone voltage 120 V. Lockmass corrections, using $[M+H]^+$ ion at 556.2771 of a Leucine Enkephalin solution, were applied for accurate mass measurements (elemental composition determination). For MS/MS experiments, argon was used as collision gas, and the collision energy was set to 110 eV for $[M+Na]^+$ precursor ion at m/z 2019.6. The mass range was 50–2500 Da and spectra were recorded at 1 s/scan in the profile mode at a resolution of 35,000 (FWHM). Data acquisition and processing were performed with MassLynx 4.1 software.

All NMR experiments presented for compound **3** were performed at 600.17 MHz using

[1] (*a*) N. Masurier, F. Estour, M.T. Froment, B. Lefèvre, J.C. Debouzy, B. Brasme, P. Masson, O. Lafont, *Eur. J. Med. Chem.*, 2005, **40**, 615-623; (*b*) S. Graßmann, J. Apelt, W. Sippl, X. Ligneau, H.H. Pertz, Y.H. Zhao, J.M. Arrang, C. R. Ganellin, J.C. Schwartz, W. Schunack, Holger Stark, *Bioorg. & Med. Chem.*, 2003, **11**, 2163-2174; (*c*) R. Houssin, J. Pommery, M.C. Salaün, S. Deweer, J.F. Goossens *J. Med. Chem*., 2002, **45**, 533-536.

Bruker Avance III spectrometer (Wissembourg, France) with a 5mm triple resonance probe TXI (HCN) equipped with Z-gradient coil for pulsed-field gradient spectroscopy. The calibration of spectra was performed using the signal of the residual protons and carbons of $CDCl_3$. Measurements were performed at 300K with careful temperature regulation. Details concerning the type of NMR experiment will be given in the figure captions.

Routine HRMS, NMR ^1H and ^{13}C NMR spectra obtained in the case of β-cyclodextrin derivatives **2**, **4, 5, 9b** and **9** are given. ESI-MS spectra were obtained with a HCT Ultra Ion Trap mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with an electronspray source. NMR spectra were recorded on a Bruker Avance 300 instrument spectrometer. Chemical shift (δ) values are given in ppm and coupling constants (*J*) are given in Hertz. All NMR experiments were performed (^1H at 300 MHz, ^{13}C at 75 MHz) in $CDCl_3$ at 300 K. The ^{13}C signals are given with one decimal except when they are too close (two decimals).

The degradation curves of soman by 2-iodosobenzoic acid or compounds **4**, **5** or **9** were obtained by 1D ^1H NMR experiments on a Bruker Avance 600 instrument spectrometer equipped with a triple cryoprobe CPTCI (^1H, ^{31}P, ^{13}C). One dimensional ^1H NMR spectra were recorded at 25°C with water signal suppression (pre-saturation and excitation sculpting), during a total acquisition time of 1 min 30 sec (number of scans equal to 16 and a presaturation delay of 3 sec).

Ellman solution for the detoxification assays of soman by 2-iodosobenzoic acid or compounds **4**, **5** or **9** was prepared in dissolving of acetylthiocholine (200 mg), 5,5'-dithiobis-(2-nitrobenzoic acid) (100mg), and sodium bicarbonate (50mg) in 25mL of phosphate buffer (pH_7). A ten-fold dilution of this solution was then carried out. Acetylcholinesterase (AChE) from human erythrocytes was purchased from Sigma Aldrich. The residual AChE activity was determined on a microplate reader PowerWave XS® (Biotek).

2. Experimental procedures for the synthesis of compounds 2 5

2.1 Synthesis of compound 2

The synthetic pathway is detailed in the manuscript.

2^B-O-(benzyl)-3^A-({1-trityl-1H-imidazol-4-yl}methyl)-2^A,3^B,6^A,6^B-tetra-O-methyl-pentakis-(2,3,6-tri-O-methyl)-cyclomaltoheptaose 7: NaH (60% in mineral oil, 0.158 g, 3.95 mmol) was added to a solution of compound **6** (1.96 g, 1.31 mmol) in 16.8 mL of anhydrous dimethyl sulfoxide at room temperature under argon. The reaction mixture was stirred at room temperature for 7 hours. 4-(chloromethyl)-1-trityl-1*H*-imidazole (1.42 g, 3.95 mmol) in 5.1 mL of anhydrous dimethyl sulfoxide was added. The reaction mixture was stirred at room temperature overnight. Water (15 mL) was added dropwise to quench the reaction. The reaction mixture was extracted with ethyl acetate (3 x 50 mL). The organic layers were collected; they were washed with 1N aqueous solution of HCl (2 x 10 mL), then brine (10 mL), and dried over magnesium sulfate. After filtration, the solvent was removed under reduced pressure. The residue was purified to flash chromatography on silica gel (dichloromethane/ethyl acetate/methanol, 5/5/0.2; v/v/v) to give the desired product (m=1.30g). White powder, 70% yield; mp > 260°C; IR ν_{max}/cm^{-1} 2925; ^1H NMR ($CDCl_3$) δ 3.08-3.13 (m, 6H, 6 x H-2), 3.21 (m, 3H, 1 x $OCH_{3(6)}$), 3.27 (m, 3H, 1 x $OCH_{3(6)}$), 3.29-3.58 (m, 72H, 5 x $OCH_{3(6)}$, 1 x H-2, 6 x $OCH_{3(2)}$, 6 x $OCH_{3(3)}$, 6 x H-3, 7 x H-4, 7 x H-6), 3.62-3.86 (m, 15H, 7 x H-5, 7 x H-6, 1 x H-3), 4.32-4.42 (m, 2H, CH_2-Im), 4.69 (d, ^3J=3.0 Hz, 1H, H-1), 4.75 (d, ^2J= 15.0 Hz, 1H, CH_{2b}-Ph), 5.01-5.11 (m, 6H, 6 x H-1), 5.27 (d, ^3J=15.0 Hz, 1H, CH_{2a}-Ph), 6.85 (s, 1H, H-arom), 6.94-6.97 (m, 6H, H-arom), 7.09-7.32 (m, 15H, H-arom); ^{13}C NMR ($CDCl_3$): δ 58.0, 58.2, 58.3, 58.4, 58.5, 58.8, 58.9, 59.0, 59.4 [13C, 6 x OCH_3(C-2), 7 x OCH_3(C-6)], 60.8, 61.0, 61.1, 61.2, 61.3, 61.5, 61.6, 61.8 [6C, 2 x OCH_3(C-3)], 64.7 (OCH_2-Im), 70.4, 70.5, 70.6, 71.1, 71.5, 71.8 [14C, 7 x C-6, 7 x C-5], 74.2 (OCH_2-Ph), 78.0, 79.1, 79.4, 79.6, 79.9, 80.5, 80.8, 81.0, 81.4, 81.5, 81.8, 82.0, 82.2 [22C, 7 x C-2, 7 x C-3, 7 x C-4, C_{IV}-trityl], 97.9, 98.4, 98.6, 98.7, 99.0, 99.2 [7C, 7 x C-1], 115.9, 127.0, 127.7, 127.8, 128.4, 128.7, 128.8, 129.4, 130.6, 136.0, 138.3, 140.5, 146.9 [27C, C-arom]; ESI-MS (m/z): 1814 $[M+H]^+$; anal. calcd for $C_{91}H_{132}O_{35}N_2$: C, 60.25; H, 7.33; N, 1.54; found: C, 59.28; H, 7.29; N, 2.11.

2^B-O-hydroxy-3^A-({1-trityl-1H-imidazol-4-yl}methyl)-2^A,3^B,6^A,6^B-tetra-O-methyl-pentakis-(2,3,6-tri-O-methyl)-cyclomaltoheptaose 8:

Debenzylation step

Pd/C (10%, 0.287 g, 0.276 mmol) was added to a solution of compound **7** (0.500 g, 0.276 mmol) in 21 mL of methanol. The suspension was purged three times with nitrogen and then subjected to a flow of hydrogen. The reaction mixture was stirred at room temperature for 16 hours. The suspension was filtered through celite and washed with methanol. The filtrate was concentrated to dryness. The residue was purified to flash chromatography on silica gel (dichloromethane/ethyl acetate/methanol, 5/5/0.2 (v/v/v) to give the desired product as a white powder (0.245 g, 60% yield).

Tritylation step

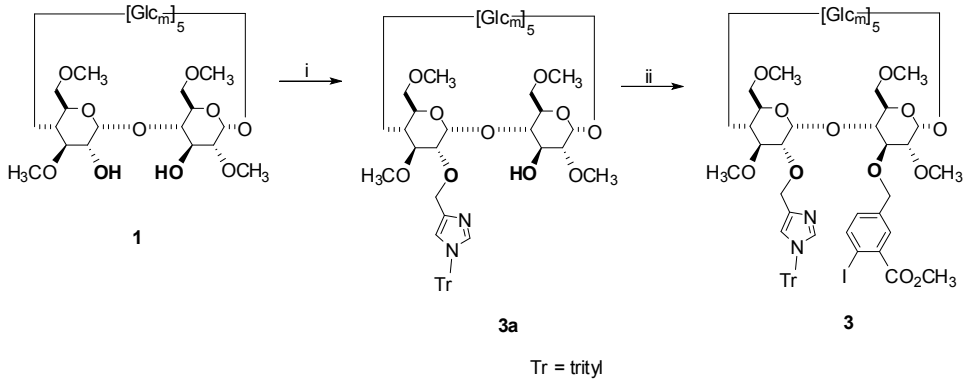
Triethylamine (0.05 mL, 0.372 mmol) was added to a solution of debenzylated compound (0.227 g, 0.153 mmol) in 1.5 mL of anhydrous dimethylformamide at room temperature under argon. A solution of trityl chloride (0.045 g, 0.164 mmol) in 1 mL of anhydrous dimethylformamide was added. The reaction mixture was stirred at room temperature overnight. Water (1 mL) was added dropwise to quench the reaction. The reaction mixture was extracted in EtOAc, washed with brine. It was dried over magnesium sulfate. After filtration, the solvent was removed under reduced pressure. The residue was purified to flash chromatography on silica gel (dichloromethane/ethyl acetate/methanol, 5/5/0.6; v/v/v) to give the desired product (m=0.170 g). White powder, 65% yield; mp > 260°C; IR ν_{max}/cm^{-1} 2890, 2825, 1446, 1020; ^{1}H NMR ($CDCl_3$) δ 3.18-3.22 (m, 5H, 5 x H-2), 3.29 (dd, ^3J=3.0 Hz, ^3J=9.0 Hz, 1H, H-2), 3.40 (m, 19H, 6 x $OCH_{3(6)}$, H-2), 3.44-4.01 (m, 74H, 1 x $OCH_{3(6)}$, 6 x $OCH_{3(2)}$, 6 x $OCH_{3(3)}$, 7 x H-3, 7 x H-4, 14 x H-6, 7x H-5), 4.58 (d, 1H, ^2J=12.0 Hz, CH_{2a}-Im), 4.93 (d, 1H, ^2J=12.0 Hz, CH_{2b}-Im), 5.10-5.15 (m, 5H, H-1), 5.19 (s, 1H, ^3J=3.0 Hz, H-1), 5.27 (d, ^3J=3.0 Hz, 1H, H-arom), 6.79 (s, 1H, H-arom), 7.10-7.13 (m, 6H, H-arom), 7.31-7.35 (m, 9H, H-arom), 7.39 (s, 1H, H-arom); ^{13}C NMR ($CDCl_3$): δ 57.5, 58.0, 58.2, 58.4, 58.5, 58.6, 59.00, 59.0, 59.1 [13C, 6 x OCH_3(C-2), 7 x OCH_3(C-6)], 61.4, 61.5, 61.6, 61.8, 61.9, 62.2, [6C, 6 x OCH_3(C-3)], 68.9 (CH_2-Im), 70.6, 70.7, 70.9, 71.0, 71.2, 71.3, 71.4, 71.6, 72.2 [14C, 7 x C-5, 7 x C-6], 75.5 (C_{IV}-trityl), 80.0 80.3, 80.7, 80.8, 80.9, 81.2, 81.4, 81.6, 81.7, 81.8, 81.9, 82.0, 82.1, 82.3, 82.9 [21C, 7 x C-2, 7 x C-3, 7 x C-4],

98.4, 98.7, 98.9 99.0, 99.1, 99.3, 99.4 [6C, 6 x C-1], 101.5 (C-1) 120.0, 128.1, 129.8, 137.9, 139.2, 142.2 [21C, C-arom]; ESI-MS (m/z): 1723 $[M+H]^+$; anal. calcd for $C_{84}H_{126}N_2O_{35}$: C, 58.52; H 7.37, N, 1.63; found: C, 58.46, H, 7.29, N, 1.98.

2^B-O-(3-carboxymethyl-4-iodobenzyl)-3^A-({1-trityl-1H-imidazol-4-yl}methyl)-O-2^A,3^B, 6^A,6^B-tetra-O-methyl-pentakis-(2,3,6-tri-O-methyl)-cyclomaltoheptaose 2: NaH (60% in mineral oil, 0.028 g, 0.697 mmol) was added to a solution of compound **8** (0.4 g, 0.232 mmol) in 3 mL of anhydrous dimethyl sulfoxide at room temperature under argon. The reaction mixture was stirred at room temperature for 7 hours. Methyl 5-bromomethyl-2-iodobenzoate (0.247 g, 0.697 mmol) in 0.9 mL of anhydrous dimethyl sulfoxide was added. The reaction mixture was stirred at room temperature overnight. Water (5 mL) was added dropwise to quench the reaction. The reaction mixture was extracted with ethyl acetate (3 x 50 mL). The organic layers were collected; they were washed with 1N aqueous solution of HCl (2 x 10 mL), then brine (10 mL), and dried over magnesium sulfate. After filtration, the solvent was removed under reduced pressure. The residue was chromatographied on silica gel (dichloromethane/ethyl acetate/methanol, 5/5/0.5; v/v/v) to give the desired product (m=0.120 g). White powder, 26% yield; mp > 260°C; IR ν_{max}/cm^{-1} 2925, 2830, 1735; ^1H NMR ($CDCl_3$) δ 3.17-3.25 (m, 6H, 5 x H-2, H-2A), 3.30 (m, 3H, 1 x $OCH_{3(6)}$), 3.34 (m, 3H, 1 x $OCH_{3(6)}$), 3.38 (m, 15H, 5 x $OCH_{3(6)}$), 3.45-3.92 (m, 75H, H-2B, 6 x $OCH_{3(2)}$, 6 x $OCH_{3(3)}$, 7 x H-3, 7 x H-4, 14 x H-6, 7 x H- 5, $COOC\underline{H}_3$), 4.36-4.49 (m, 2H, CH_2-Im), 4.73-4.88 (m, 2H, CH_2-Ph), 5.11-5.16 (m, 7H, H-1), 6.83 (s, 1H, H-arom), 7.05-7.10 (m, 6H, H-arom), 7.44 (d, ^3J=6.0 Hz, 1H, H-arom), 7.26-7.35 (m, 10H, H-arom), 7.72 (s, 1H, H-arom), 7.83 (d, ^3J=9.0 Hz, 1H, H-arom); ^{13}C NMR ($CDCl_3$): δ 52.4 ($COO\underline{C}H_3$), 58.4, 58.50, 58.53, 58.6, 58.7, 58.94, 58.97, 58.98, 59.01, 59.04 [13C, 6 x OCH_3(C-2), 7 X OCH_3(C-6)], 61.38, 61.43, 61.5, 61.57, 61.63, 61.8 [6C, 6 x OCH_3(C-3)], 70.79, 70.82, 70.87, 70.99, 71.06, 71.36, 71.39, 71.46, 71.52, 71.7 [16C, 7 x C-5, 7 x C-6, OCH_2-Ph, OCH_2-Im], 75.4 (C_{IV}-Tr), 79.6, 79.8, 80.0, 80.4, 80.5, 80.6, 81.77, 81.83, 81.90, 81.95, 82.03, 82.05, 82.1, 82.2, 82.3 [21C, 7 x C-2, 7 x C-3, 7 x C-4], 92.8 (C-I), 98.94, 99.00, 99.04, 99.08 [7 C, 7 x C-1], 119.5, 128.0, 129.7, 130.3, 132.3, 134.6, 138.5, 139.3, 141.3, 142.4, [26C, C-arom], 166.7 (CO); ESI-MS (m/z): 1997 $[M+H]^+$; HMRS calcd for $C_{93}H_{133}IN_2O_{37}$: 1997.7710, found: 1997.7703; anal. calcd for $C_{93}H_{133}IN_2O_{37}$: C, 55.91; H, 6.71; N, 1.40; found: C, 55.36; H, 6.72; N, 1.84.

2.2 Synthesis of compound 3

Compound **3** was synthetized according to the following pathway.



Reagents and conditions: i) 1. NaH, DMSO, Ar; 2. 4-(chloromethyl)-1-trityl-1*H*-imidazole. ii) 1. NaH, DMSO, Ar; 2. Methyl 5-(bromomethyl)-2-iodobenzoate.

2^B-O-({1-trityl-1H-imidazol-4-yl}methyl)-3^A-hydroxy-2^A,3^B,6^A,6^B-tetra-O-methyl-pentakis-(2,3,6-tri-O-methyl)-cyclomaltoheptaose 3a: NaH (60% in mineral oil) (0.014 g, 0.357 mmol) was added to a solution of compound **1** (0.500 g, 0.357 mmol) in 10 mL of anhydrous dimethyl sulfoxide at room temperature under argon. The reaction mixture was stirred at room temperature for 7 hours. 4-(chloromethyl)-1-(triphenylmethyl)-imidazole (0.128 g, 0.357 mmol) was then added. The reaction mixture was stirred at room temperature overnight. Water (5 mL) was added dropwise to quench the reaction. The reaction mixture was extracted with ethyl acetate (3 x 40 mL). The organic layer was washed with brine and it was dried over magnesium sulfate. After filtration, the solvent was removed under reduced pressure. The residue was chromatographied on silica gel (dichloromethane/ethyl acetate/methanol, 5/5/0.6; v/v/v) to give the desired product (m= 0.31 g). White powder, 50% yield; mp > 260°C; IR ν_{max}/cm^{-1} 2892, 2823, 1448, 1022; ^{1}H NMR ($CDCl_3$) δ 3.08 (dd, ^3J=9.0 Hz, ^3J=3.0 Hz, 1H, 1 x H-2), 3.14-3.19 (m, 5H, 5 x H-2), 3.34-3.39 (m, 22H, 1 x H-2, 7 x $OCH_{3(6)}$), 3.43-3.63 (56H, 6 x $OCH_{3(2)}$, 6 x $OCH_{3(3)}$, 6 x H-3, 7 x H-4, 7 x H-6), 3.66-3.86 (m, 14H, 7 x H-5, 7 x H-6), 3.92 (t, ^{3}J= 9.0 Hz,1H, 1 x H-3), 4.64-4.75 (AB spectrum, 2H, ^2J= 12.0Hz, CH_2-Im,), 4.77 (d, ^3J= 3.0 Hz, 1H, 1 x H-1), 5.04 (d, ^3J= 3.0 Hz, 1H, 1 x H-1), 5.07-5.13 (m, 5H, 5 x H-1), 5.34 (bs, 1H, OH), 6.89 (s, 1H, H-arom), 7.09-7.12 (m, 6H, H-arom), 7.29-7.34 (m, 9H, H-arom), 7.36 (s, 1H, H-arom); ^{13}C NMR ($CDCl_3$): δ 58.4, 58.5, 58.7, 59.00, 59.03, 59.06 [13C, 6 x OCH_3(C-2), 7 x OCH_3(C-6)], 61.47, 61.49, 61.6, 61.70, 61.73, 61.9 [6C, 6 x OCH_3(C-3)], 70.2, 70.9, 71.01, 71.06, 71.15, 71.3, 71.5, 71.6 [15C, O$\underline{C}H_2$-Im, 7 x C-5, 7 x C-6], 71.8 (C-3), 75.5 (C_{IV}-trityl), 79.2

(C-2), 80.1, 80.3, 80.5, 81.1, 81.29, 81.35, 81.57, 81.60, 81.7, 81.80, 81.84, 82.1, 82.2, 82.4, 82.5, 83.2 [19C, 6 x C-2, 6 x C-3, 7 x C-4], 99.0, 99.1, 99.4, 99.5, 99.9 [6C, 6 x C-1], 101.4 (C-1), 121.4, 128.2, 129.8, 137.6, 138.8, 142.3 [21C, C-arom]; ESI-MS (m/z): 1723 $[M+H]^+$; anal. calcd for $C_{84}H_{126}N_2O_{35}$: C, 58.52; H 7.37, N, 1.63; found: C, 58.46, H, 7.29, N, 1.98.

2^B-O-({1-trityl-1H-imidazol-4-yl}methyl)-3^A-O-(3-carboxymethyl-4-iodobenzyl)-2^A,3^B,6^A,6^B-tetra-O-methyl-pentakis-(2,3,6-tri-O-methyl)-cyclomaltoheptaose 3: NaH (60% in mineral oil) (0.028 g, 0.697 mmol) was added to a solution of compound **3a** (0.4 g, 0.232 mmol) in 3 mL of anhydrous dimethyl sulfoxide at room temperature under argon. The reaction mixture was stirred at room temperature for 7 hours. Methyl 5-bromomethyl-2-iodobenzoate (0.247 g, 0.697 mmol) in 0.9 mL of dimethyl sulfoxide was added. The reaction mixture was stirred at room temperature overnight. Water (5mL) was added dropwise to quench the reaction. The reaction mixture was extracted in ethyl acetate (3 x 30mL). The organic layers were collected; they were washed with 1N aqueous solution of HCl (2 x 10 mL), then brine (10 mL), and dried over magnesium sulfate. After filtration, the solvent was removed under reduced pressure. The residue was chromatographied (dichloromethane/ethyl acetate/methanol, 5/5/0.5; v/v/v) to give the desired product (m=0.186 mg). White powder, 40% yield; mp > 260°C; IR ν_{max}/cm^{-1} 2924, 2831, 1734; ^{1}H NMR ($CDCl_3$) δ 3.15-3.21 (m, 6H, 5 x H-2, H-2A), 3.32 (m, 3H, 1x $OCH_{3(6)}$), 3.39-3.40 (m, 18H, 6 x $OCH_{3(6)}$), 3.47-4.01 (m, 75H, H-2B, 6 x $OCH_{3(2)}$, 6 x $OCH_{3(3)}$, 7 x H-3, 7 x H-4, 7 x H-5, 14 x H-6, $COOOCH_3$), 4.50-4.60 (m, 2H, CH_2-Im), 4.63 (d, ^3J=12.0 Hz, 1H, CH_{2a}-Ph), 5.03-5.04 (d, ^3J=3.0Hz, 1H, H-1), 5.10-5.19 (m, 7H, 6 x H-1, CH_{2b}-Ph), 6.80 (s, 1H, H-Im), 7.06-7.09 (m, 6H, H-Ph_{Tr}), 7.21-7.24 (dd, ^{3}J=6.0 Hz, ^4J=3.0 Hz, 1H, H-Ph), 7.29-7.31 (m, 9H, H-Ph_{Tr}), 7.35 (s, 1H, H-Im), 7.79 (d, ^3J=6.0 Hz, 1H, H-Ph), 7.85 (d, ^4J=3.0 Hz, 1H, H-Ph); ^{13}C NMR ($CDCl_3$): δ 52.3 ($COOCH_3$), 58.4, 58.5, 58.7, 58.8, 58.9, 58.99, 59.01, 59.06, 59.07 [13C, 6 x OCH_3(C-2), 7 X OCH_3(C-6)], 61.3, 61.4, 61.6, 61.7 [6C, 6 x OCH_3 (C-3)], 66.8 (OCH_2-Im), 70.80, 70.85, 70.96, 71.09, 71.11, 71.3, 71.6, 71.8 [14C, 7 x C-5, 7 x C-6], 74.4 (OCH_2-Ph), 75.3 (C_{IV}-Tr), 79.5, 79.8, 79.98, 80.07, 80.20, 80.26, 80.6, 80.72, 80.76, 81.5, 81.79, 81.84, 81.88, 81.91, 82.00, 82.09, 82.12, 82.21, 82.26, 82.32 [21C, 7 x C-2, 7 x C-3, 7 x C-4], 92.1 (C-I), 98.95, 99.00, 99.08, 99.13 [7 C, 7 x C-1], 120.5, 128.1, 129.8, 130.6, 132.5, 134.3, 138.61, 138.68, 140.3, 140.9,

142.5 [26C, C-arom]167.0 (CO); ESI-MS (m/z): 1997 $[M+H]^+$; HMRS calcd for $C_{93}H_{133}IN_2O_{37}$: 1997.7710, found: 1997.7753; anal. calcd for $C_{93}H_{133}IN_2O_{37}$: C, 55.91; H, 6.71; N, 1.40; found: C, 55.36; H, 6.72; N, 1.84.

2.3 Synthesis of scavengers 4 and 5

General procedure

Compound **2** or **3** (0,1g, 0.050 mmol) was solubilized in a solution of acetic acid (30% in water; v/v) (1.14 mL). Sodium periodate (1.07 g, 5.0 mmol) was then added. The mixture is stirred 24 hours at 45°C. After cooling to room temperature, the mixture was filtered to remove the excess of sodium periodate. The filtrate was extracted with dichloromethane (3 x 30 mL) and diethyl ether (2 x 20 mL). The combined organic layers were dried over magnesium sulfate. After filtration, the solvent was removed under reduced pressure. The crude product is chromatographied on reversed phase (gradient: water, 100% to water/acetonitrile, 70/30; v/v) to give the desired product.

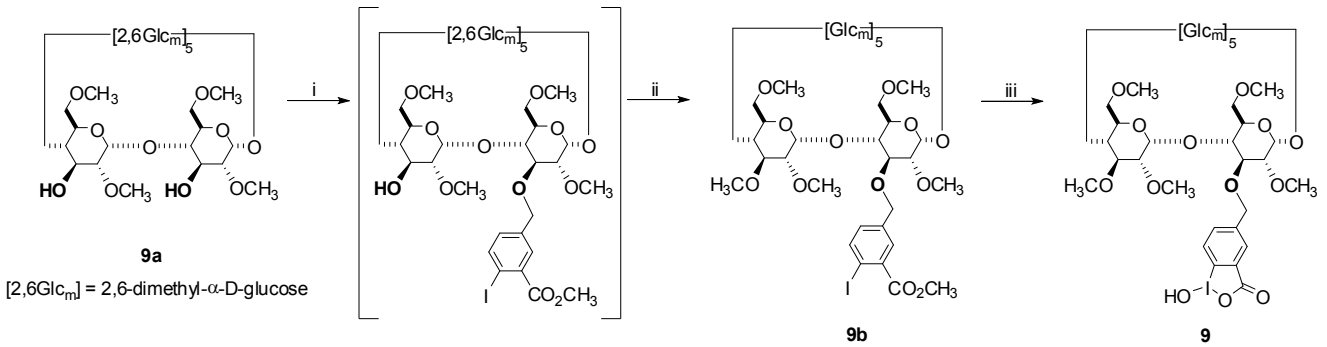
2^B-O-(3-carboxy-4-iodosobenzyl)-3^A-O-({1H-imidazol-4-yl}methyl)-2^A,3^B,6^A,6^B-tetra-O-methyl-pentakis-(2,3,6-tri-O-methyl)-cyclomaltoheptaose 4: White powder, 65% yield; mp > 260°C; IR ν_{max}/cm^{-1} 3438, 2929, 1647, 1457, 1041; ^1H NMR ($CDCl_3$) δ 3.17-3.21 (m, 6H, 5 x H-2, H-2A), 3.39 (m, 21H, 7 x $OCH_{3(6)}$), 3.48-3.93 (m, 72H, H-2B, 6 x $OCH_{3(2)}$, 6 x $OCH_{3(3)}$, 7 x H-3, 7 x H-4, 7 x H-5, 14 x H-6), 4.73-4.98 (m, 4H, CH_2-Im, CH_2-Ph), 5.08-5.25 (m, 7H, H-1), 6.74 (s, 1H, H-arom), 7.60 (s, 1H, H-arom), 7.81 (s, 2H, H-arom), 8.17 (s, 1H, H-arom); ^{13}C NMR ($CDCl_3$): δ 58.1, 58.1, 58.4, 58.5, 58.7, 58.9, 59.0, 59.1, [13C, 6 x OCH (C-2), 7 x OCH_3(C-6)], 61.0, 61.1, 61.3, 61.6, 61.7 [6C, 6 x OCH_3(C-3)], 66.3 (OCH_2-Im), 70.6, 70.9, 71.1, 71.5, 71.7 [15 C, 7 x C-5, 7 x C-6, OCH_2-Ph], 74.9 (OCH_2-Ph), 78.8, 79.2, 80.1, 80.4, 80.8, 81.5, 81.7, 81.8, 82.0 [21C, 7 x C-2, 7 x C-3, 7 x C-4], 97.9, 98.8, 98.9, 99.0 [7 C, C-1], 119.8 (C-IO), 125.2, 126.2, 128.2, 129.0, 130.8, 131.1, 133.5, 140.6 [8C, C-arom], 169.3 (CO); ESI-MS (m/z): 1757 $[M+H]^+$, 1779 $[M+Na]^+$, 1795 $[M+K]^+$; HMRS calcd for $C_{73}H_{118}IN_2O_{38}$: 1757.6407, found: 1757.6409.

2^B-O-({1H-imidazol-4-yl}methyl)-3^A-O-(3-carboxy-4-iodosobenzyl)-2^A,3^B,6^A,6^B-tetra-O-methyl-pentakis-(2,3,6-tri-O-methyl)-cyclomaltoheptaose 5: White powder, 58% yield; mp > 260°C; IR ν_{max}/cm^{-1} 3441, 2935, 1649, 1453, 1043; ^1H NMR ($CDCl_3$) δ 3.14-3.22 (m, 6H, 5 x H-2, H-2A), 3.36 (m, 21H, 7 x $OCH_{3(6)}$), 3.41-3.94 (m, 72H, H-2B, 6 x $OCH_{3(2)}$, 6 x $OCH_{3(3)}$, 7 x H-3, 7 x H-4, 7 x H- 5, 14 x H-6), 4.45-4.60 (m,

2H, CH_2-Im), 4.83-5.02 (m, 2H, CH_2-Ph), 5.05-5.29 (m, 7H, H-1), 6.81 (s, 1H, H-arom), 7.52 (s, 1H, H-arom), 7.75-7.86 (m, 2H, H-arom), 8.18 (s, 1H, H-arom); ^{13}C NMR ($CDCl_3$): δ 58.1, 58.45, 58.48, 58.5, 58.83, 58.87, 58.90, 58.91, 58.96 [13C, 6 x OCH_3(C-2), 7 x OCH_3(C-6)], 61.1, 61.3, 61.4, 61.52, 61.55 [6C, 6 x OCH_3(C-3)], 64.2 (OCH_2-Im), 70.7, 70.9, 71, 71.1, 71.2, 71.4, 71.52, 71.57 [14C, 7 x C-5, 7 x C-6], 74.8 (OCH_2-Ph), 79.3, 79.8, 80.1, 80.4, 80.6, 80.8, 81.6, 81.70, 81.74, 81.75, 81.8, 81.94, 81.99, 82.1 [21C, 7 x C-2, 7 x C-3, 7 x C-4], 98.44, 98.47, 98.7, 98.81, 98.83, 99.03, 99.08 [7C, C-1], 119.3 (C-IO), 125.85, 125.88, 128.1, 128.9, 130.7, 131.1, 134.1, 142.0 [8C, C-arom], 169.6 (CO); ESI-MS (m/z): 1757 $[M+H]^+$, 1779 $[M+Na]^+$, 1795 $[M+K]^+$; HMRS calcd for $C_{73}H_{118}IN_2O_{38}$: 1757.6407, found: 1757.6376.

2.4 Synthesis of scavenger 9

Compound **9** was synthetized according to the following pathway.



Reagents and conditions: i) 1. NaH, DMSO, Ar; 2. Methyl 5-(bromomethyl)-2-iodobenzoate. ii) 1. NaH, DMSO, Ar; 2. CH_3I, overnight. iii) 1. $NaIO_4/CH_3CO_2H$, H_2O, 45°C, 24h.

Compound **9a** was obtained as already described.[2]

3^I-O-(3-carboxymethyl-4-iodobenzyl-3,6-di-O-methyl)-hexakis-(2,3,6-tri-O-methyl)-cyclomaltoheptaose 9b: To a solution of compound **9a** (0.5 g, 0.376 mmol, previously dried for 48 h under vacuum at 80°C) in 5 mL of dry DMSO, was added NaH (60% in mineral oil, 0.105 g, 2.63 mmol) and the mixture was stirred under argon for 1 hour at room temperature. A solution of methyl 2-iodo-5-bromomethylbenzoate (0.67 g, 1.88 mmol) in 1.5 mL of dry DMSO was then added and the reaction mixture was stirred for 48 hours at room temperature. DMSO (5 mL) and NaH (60% in mineral oil, 0.21 g, 5.26 mmol) were added. The reaction mixture was stirred under argon for 1 hour at room temperature. Methyl iodide (0.75 g, 5.26 mmol) was added. The mixture was stirred for overnight. The excess of NaH was quenched by methanol (5 mL) and water

[2] (*a*) J. Szejtli, A. Liptak, I. Jodal, P. Fugeli, P. Nanasi, A. Neszmelyi, *Starch/Staerke*, 1980, **32**, 165; (*b*) T. Irie, K. Fukunaga, J. Pitha, K. Uekama, H.M. Fales, E.A. Sokolowski, *Carbohydr. Res.*, 1989, **192**, 167-172.

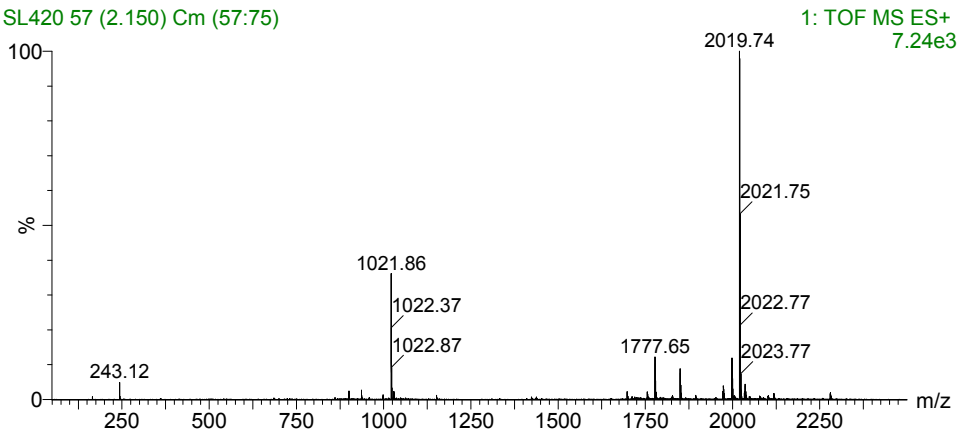
(10 mL). The reaction mixture was extracted by chloroform (2x100 mL), the combined organic layers were washed with 10% aqueous HCl solution (2x50 mL) and water (50 mL). The organic solution was separated and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (toluene-acetone, 50/50 to 75/25) to give the desired product. White powder, 8% yield; mp > 260°C; IR ν_{max}/cm^{-1} 3413, 3060, 2935, 1729; ^1H NMR ($CDCl_3$) δ 3.16-3.20 (m, 7H, 7 x H-2), 3.38 (s, 21H, 7x $OCH_{3(6)}$), 3.46-3.64 (m, 60H, 7 x H-3, 7 x H-4, 7 x H-5, 7x $OCH_{3(2)}$, 6 x $OCH_{3(3)}$), 3.75-3.82 (m, 14H, 14 x H-6), 3.90 (s, 3H, $CO_2C\underline{H}_3$), 4.72 (d, 1H, ^3J=11Hz, CH_{2a}-Ph), 5.10-5.16 (m, 8H, H-1, CH_{2b}-Ph), 7.26 (m, 1H, H-arom), 7.89-7.94 (m, 2H, H-arom); ^{13}C NMR ($CDCl_3$): δ 52.4 (CO_2CH_3), 58.4, 58.5, 58.57, 58.59, 58.7, 58.8, 58.9, 58.99, 59.02, 59.1 [14C, 7 x OCH_3 (C-2), 7 x OCH_3 (C-6)], 61.36, 61.43, 61.58, 61.61 [6C, 6 x OCH_3 (C-3)], 70.82, 70.93, 70.98, 71.03, 71.15, 71.20, 71.4, 71.5 [14C, 7 x C-5, 7 x C-6], 74.4 (CH_2-Ph), 79.9, 80.1, 80.22, 80.24, 80.4, 80.5, 80.6 [7C, 7 x C-4], 81.75, 81.80, 81.82, 81.86, 81.88, 81.93, 81.96, 82.00, 82.10, 82.15, 82.20 [14C, 7 x C-2, 7 x C-3], 92.2 (C-I), 98.7, 98.99, 99.04 [7C, 7 x C-1], 130.3, 132.0, 134.6, 140.1, 141.0 [5C, C-arom], 167.0 (CO); ESI-MS (m/z): 1711 $[M+Na]^+$; anal. calcd for $C_{71}H_{117}IO_{37}$: C, 50.47; H, 6.98; found: C, 50.55; H, 7.02.

3^I-O-(3-carboxymethyl-4-iodosobenzyl-3,6-di-O-methyl)-hexakis-(2,3,6-tri-O-methyl)-cyclomaltoheptaose 9: Compound **9b** (83 mg, 0.049 mmol) was solubilized in a solution of acetic acid (30% in water; v/v) (1.5 mL). Sodium periodate (1.05 g, 4.91 mmol) was then added. The mixture is stirred 24 hours at 45°C. After cooling to room temperature, the mixture was filtered to remove the excess of sodium periodate. The filtrate was extracted with dichloromethane (3 x 30 mL) and diethyl ether (2 x 20 mL). The combined organic layers were dried over magnesium sulfate. After filtration, the solvent was removed under reduced pressure. The crude product is chromatographied on reversed phase (gradient: water, 100% to water/acetonitrile, 50/50; v/v) to give the desired product. White powder, 68% yield; mp > 260°C; IR ν_{max}/cm^{-1} 2931, 1651; ^1H NMR ($CDCl_3$) δ 3.16-3.19 (m, 6H, 6 x H-2), 3.37 (m, 22H, 7 x $OCH_{3(6)}$, 1 x H-2), 3.48-3.97 (m, 74H, 7 x H-3, 7 x H-4, 7 x H-5, 7 x $OCH_{3(2)}$, 6 x $OCH_{3(3)}$, 14 x H-6), 4.91-4.97 (m, 2H, CH_2-Ph), 5.03-5.24 (m, 7H, H-1), 6.98-7.10 (m, 1H, H-Ph), 7.73-7.95 (m, 2H, 2 x H-Ph); ^{13}C NMR ($CDCl_3$): 58.22, 58.28, 58.37, 58.46,

58.54, 58.61, 58.66, 58.68, 58.72, 58.74, 58.80, 58.85, 58.87, 58.92 [14C, 7 x OCH_3 (C-2), 7 x OCH_3 (C-6)], 61.08, 61.17, 61.23, 61.27, 61.43, 61.47 [6C, 6 x OCH_3 (C-3)], 70.67, 70.72, 70.77, 70.82, 70.87, 70.90, 71.02, 71.04, 71.08, 71.22, 71.24, 71.36, 71.40, 71.47 [14C, 7 x C-5, 7 x C-6], 73.5 (1C, CH_2Ph), 79.74, 79.97, 80.02, 80.08, 80.24, 80.33, 80.38, 80.43, 81.50, 81.55, 81.61, 81.65, 81.70, 81.75, 81.80, 81.86, 81.92, 81.94, 82.00, 82.09, 82.13 [21C, 7 x C-2, 7 x C-3, 7 x C-4], 98.70, 98.76, 98.81, 98.83, 98.89, 98.92, 98.98 [7C, 7 x C-1], 116.3 (C-IO), 125.7, 130.8, 131.6, 135.4, 140.9 [5C, C-arom], 169.0 (CO); ESI-MS (m/z): 1713 $[M+Na]^+$, HMRS calcd for $C_{70}H_{115}NaIO_{38}$: 1713.6009, found: 1713.6010.

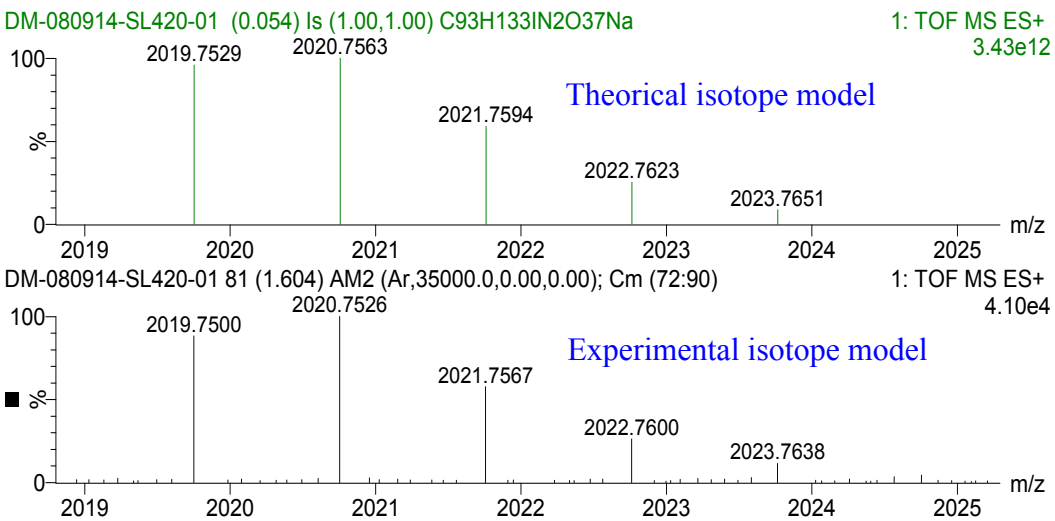
3. Detailed analysis of compound 3

3.1 ESI-HRMS spectrum of 3



Compound **3** is mainly detected on the ESI-HRMS spectrum as sodium adducts $[M+Na]^+$ and $[M+2Na]^{2+}$ at m/z 2019.7 and 1021.9, respectively.

Comparison of experimental and theorical isotope models

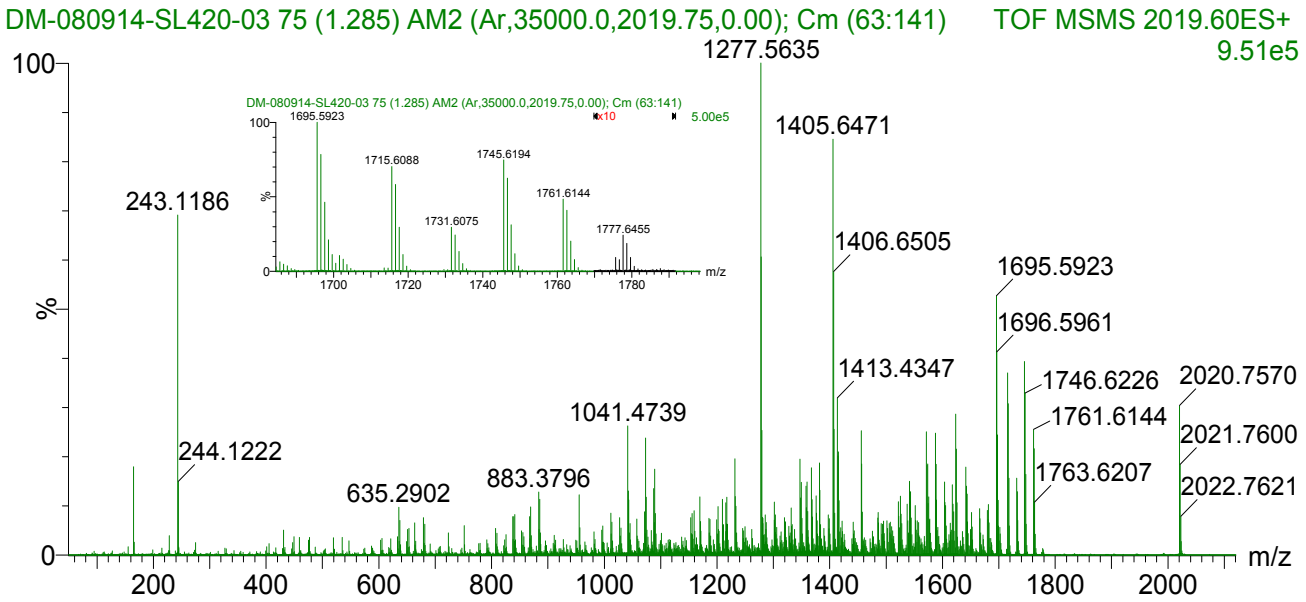


The experimental isotope model for $[M+Na]^+$ ion at m/z 2019.7 is in agreement with the theorical isotope model reconstituted from the formula $C_{93}H_{133}N_2O_{37}INa$ in terms of intensity for each isotope and mass precision.

Accurate mass measurement performed on the mono-isotopic ion at m/z 2019.7 revealed only a difference of -1.5 ppm (-3mDa) between the measured mass (2019.7500) and the calculated mass (2019.7529) which validates the formula $C_{93}H_{133}N_2O_{37}INa$ corresponding to compound **3**.

ESI-HRMS/MS spectrum of compound 3

To obtain deeper structural information, MS/MS experiments by selecting $[M+Na]^+$ ion at m/z 2019.7 and applying a collision energy of 110eV were performed.



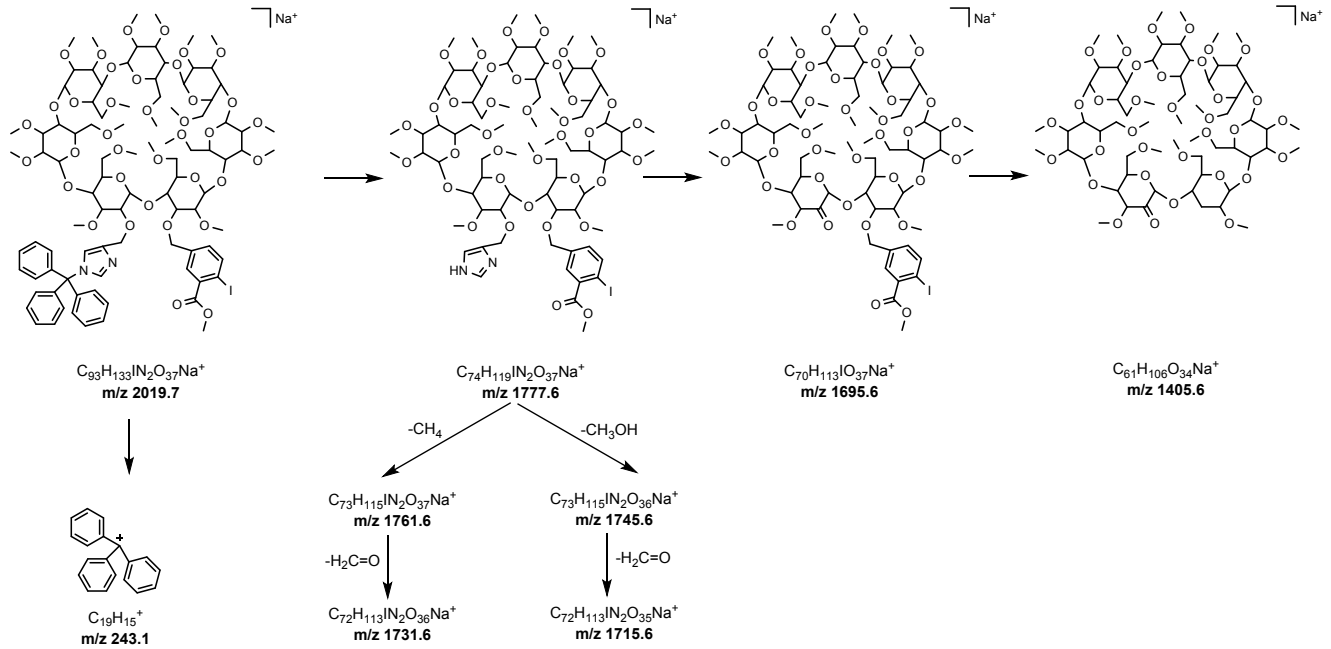
5

The accurate mass measurements results obtained for the $[M+Na]^+$ ion at m/z 2019.7 and its main fragments are collected in the table below.

Elemental compositions of the main fragments generated by MS/MS from $[M+Na]^+$ ion at m/z 2019.7

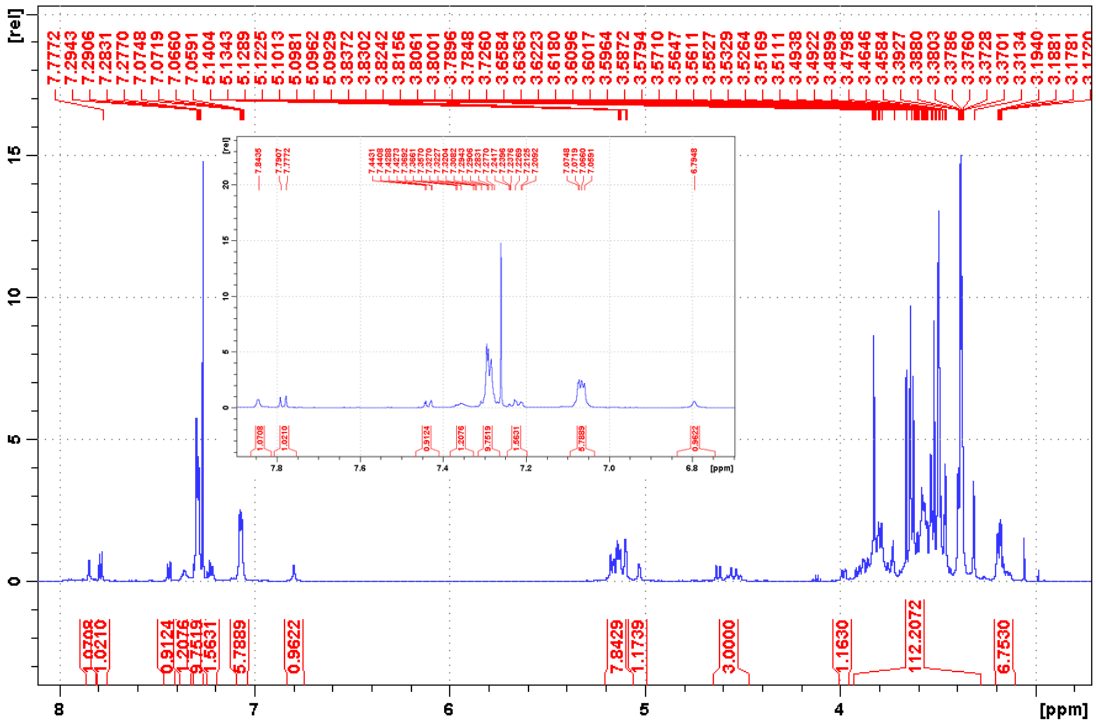
Precursor Ion (m/z)	Product Ions (m/z)	Elemental Composition	m/z (Theorical)	m/z (Experimental)	Error (ppm)
2019.7		$C_{93}H_{133}N_2O_{37}INa$	2019.7529	2019.7500	-1.5
	1777.6	$C_{74}H_{119}N_2O_{37}INa$	1777.6434	1777.6455	1.2
	1761.6	$C_{73}H_{115}N_2O_{37}INa$	1761.6121	1761.6144	1.3
	1745.6	$C_{73}H_{115}N_2O_{36}INa$	1745.6172	1745.6194	1.3
	1731.6	$C_{72}H_{113}N_2O_{36}INa$	1731.6015	1731.6075	3.5
	1715.6	$C_{72}H_{113}N_2O_{35}INa$	1715.6066	1715.6088	1.3
	1695.6	$C_{70}H_{113}O_{37}INa$	1695.5903	1695.5923	1.2
	1405.6	$C_{61}H_{106}O_{34}Na$	1405.6463	1405.6471	0.6
	1277.6	$C_{54}H_{94}O_{32}Na$	1277.5626	1277.5635	0.7
	243.1	$C_{19}H_{15}$	243.1174	243.1186	4.9

Proposed fragmentation pathway of the $[M+Na]^+$ ion at m/z 2019.7 based on MS/MS data:

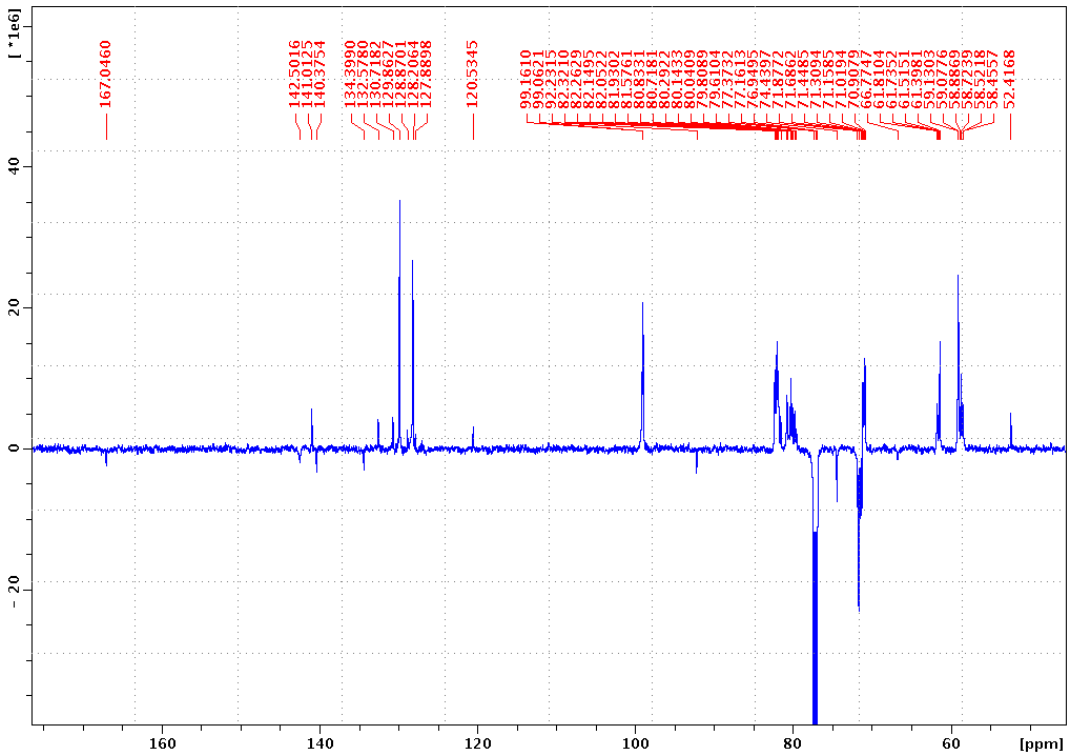


The MS/MS spectrum revealed an immediate loss of the trityl group characterized by the ion at m/z 1777.6 (very low intensity) which is also confirmed by the presence of m/z 243.1 corresponding to Ph_3C^+. The fragmentation of m/z 2019.7 and 1777.6 rises directly to ion at m/z 1695.6 clearly evidences the presence of an imidazo-trityl group in the structure of compound **3**. Moreover, the fragment ion observed at m/z 1405.6 can be explained by the loss of iodobenzoate group from the ion at m/z 1695.6. Consequently, ESI-HRMS and MS/MS confirmed the hetero difunctionalization of compound **3**. The relative position of these groups will be evidenced by NMR.

3.2 ^{1}H NMR spectrum of 3



3.3 DeptQ spectrum of 3

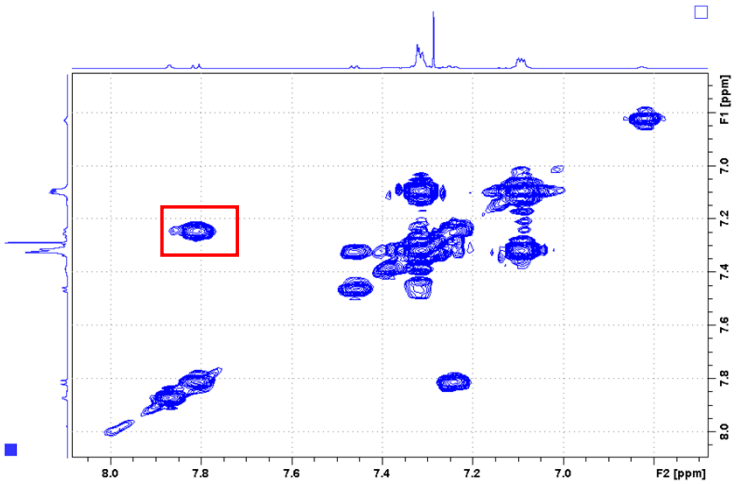


3.4 COSY, HSQC, HMBC and NOESY experiments of 3

The detailed analysis to prove the relative positions of the two substituents is based on the observed correlations between methylenic proton signals of the CH_2 groups connected to the aromatic moieties and the corresponding carbon atom signal of the substituted glucose units.

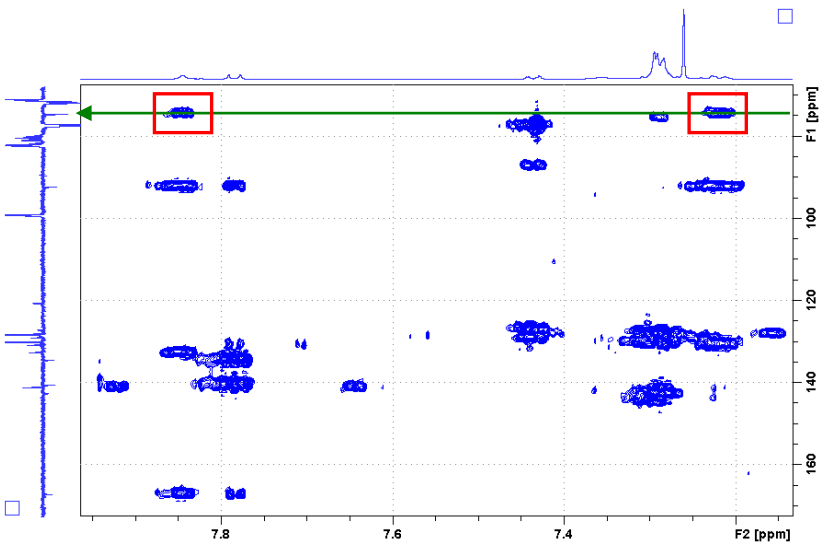
Identification of methylenic protons signals connected to aromatic moieties

The ^1H NMR revealed three distinct aromatic signals at 7.21, 7.78 and 7.84 ppm regarding the iodobenzoate moiety. The COSY experiment proved the correlation between the two doublets at 7.78 and 7.21 ppm.



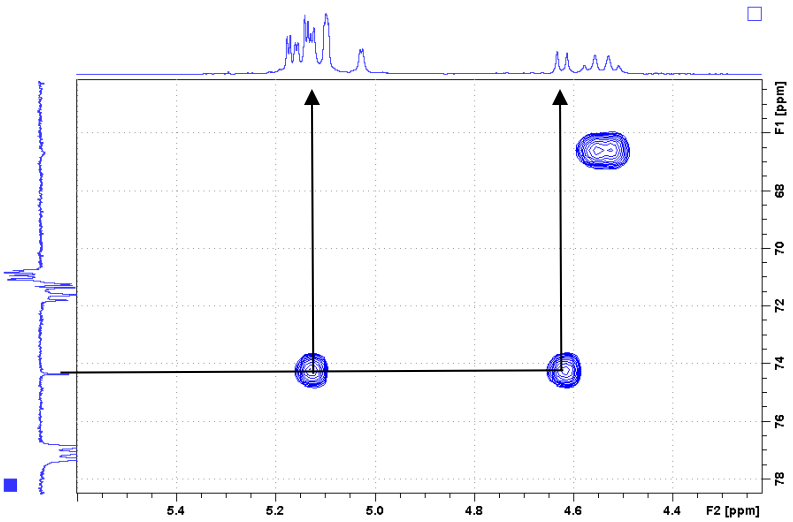
Partial contour plots of COSY experiment (area of aromatic protons)

The HMBC experiment showed two correlations between the proton signals at 7.85 and 7.21 ppm, and the same carbon signal at 74.5 ppm.



Partial contour plot of HMBC correlation (F1: DeptQ spectrum, F2: ^1H spectrum)

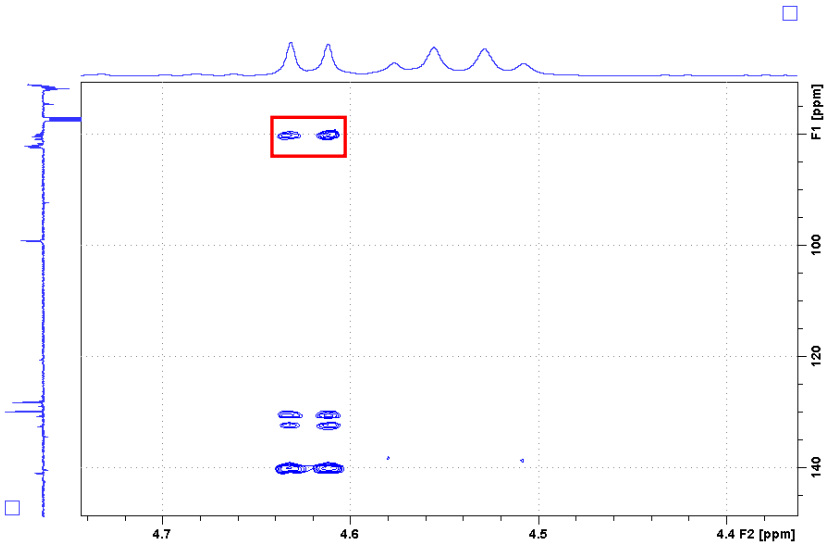
The followed partial contour plots of HSQC experiment allowed to identify the proton signals at 4.62 and 5.13 ppm of the CH_2 group connected to the benzene ring. A correlation between the protons signal at 4.51-4.56 ppm and a carbon signal at 66.8 ppm was observed in the case of imidazole substituent.



HSQC correlation experiment (F1: DeptQ spectrum, F2: ^{1}H spectrum)

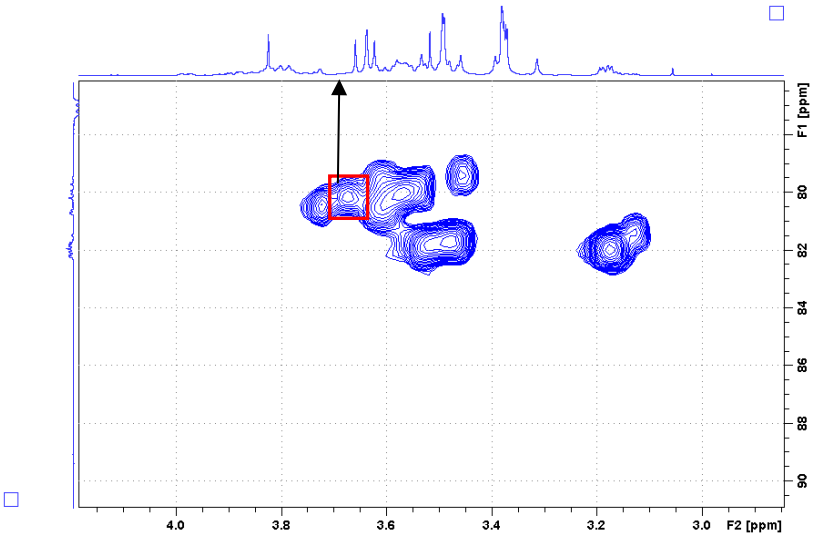
Iodobenzoate moiety at *O*-3

One of the benzylic protons signals (4.62 ppm) correlated with a carbon signal at 80.0 ppm (partial contour plot of HSQC experiment below).



Partial contour plot of HMBC correlation experiment (F1: DeptQ spectrum, F2: ^{1}H spectrum)

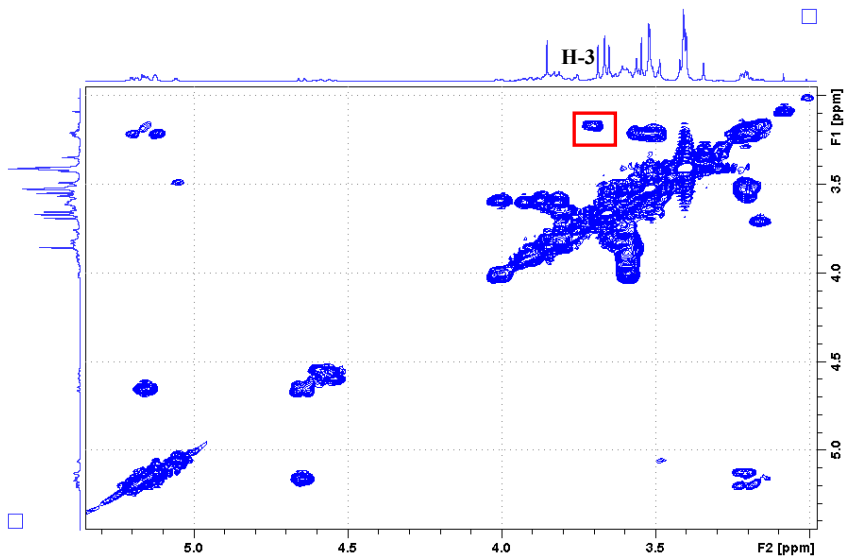
This latest signal correlated with a proton signal at 3.71 ppm (partial contour plot of HSQC experiment below).



Partial contour plot of HSQC correlation experiment

(F1: DeptQ spectrum, F2: ^{1}H spectrum)

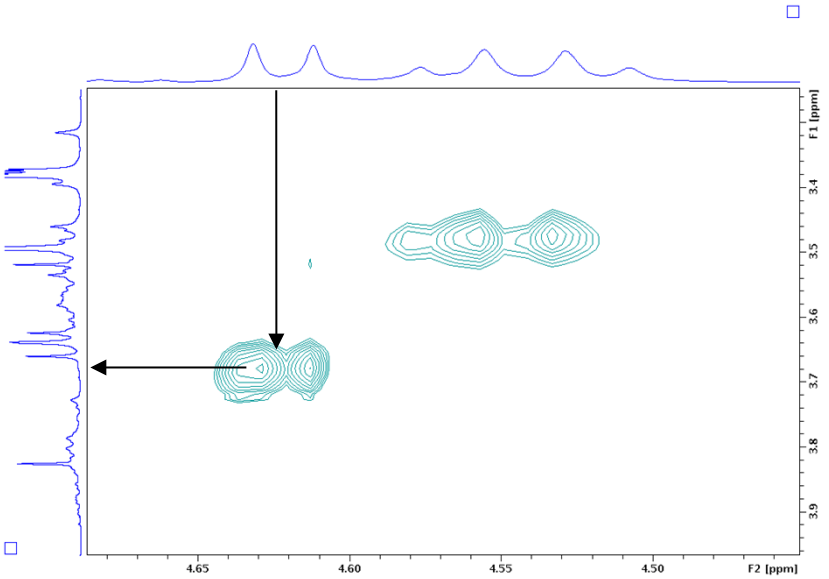
The following partial contour plot of COSY proved that it was a proton H-3.



COSY experiment

The 3-*O* substitution by the benzylic group in compound **3** was then proved by the presence of a HMBC correlation between one of the benzylic protons signal (4.62 ppm) and the carbon signal C-3 (80.0 ppm) of the glucose unit A. This result was confirmed

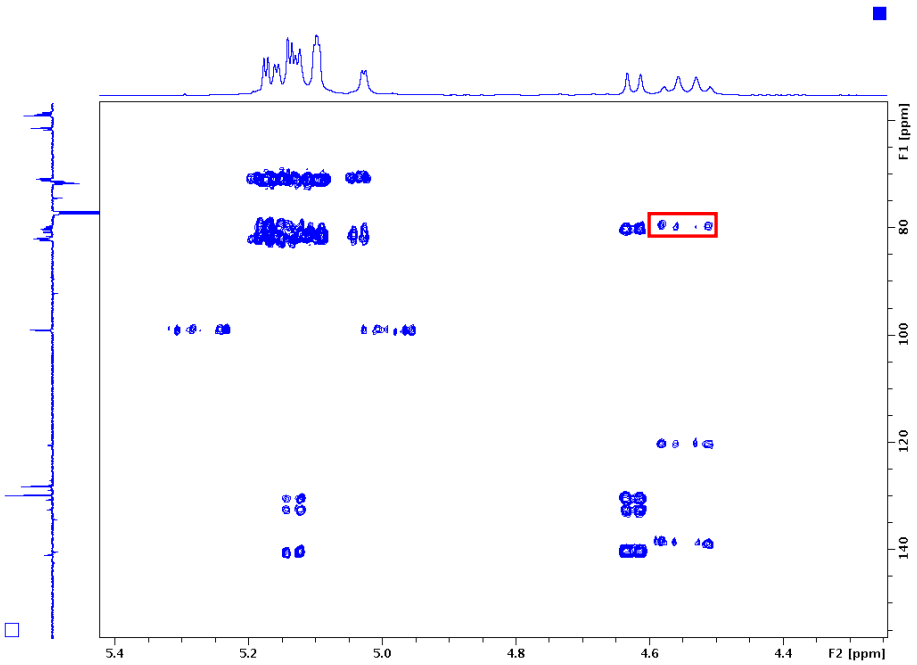
by a NOESY experiment that showed a correlation between the respective protons signals at 4.62 ppm (CH_2a-Ph) and 3.68 ppm (H-3 of the glucose unit A).



Partial contour plots of the NOESY experiment (area of benzylic protons)

Imidazole moiety at *O*-2

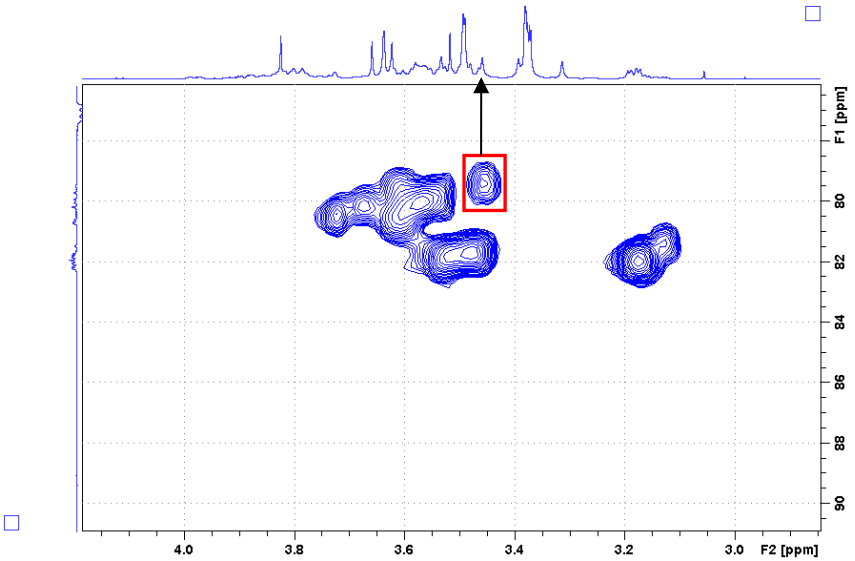
The methylenic protons signals (4.51-4.56 ppm) correlated with a carbon signal at 79.6 ppm (partial contour plot of HMBC experiment below).



Partial contour plot of HMBC correlation experiment

(F1: DeptQ spectrum, F2: ^{1}H spectrum)

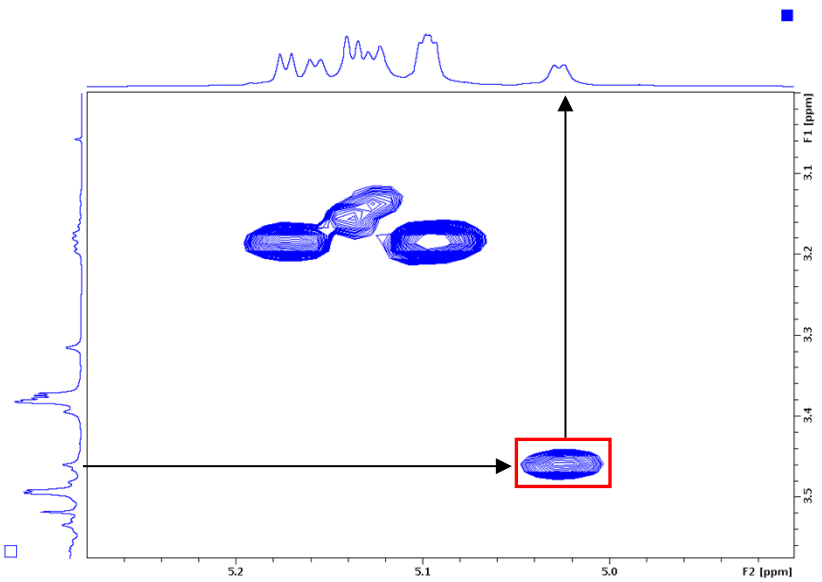
This latest signal correlated with a proton signal at 3.46 ppm (partial contour plot of HSQC experiment below).



Partial contour plot of HSQC correlation experiment

(F1: DeptQ spectrum, F2: ^{1}H spectrum)

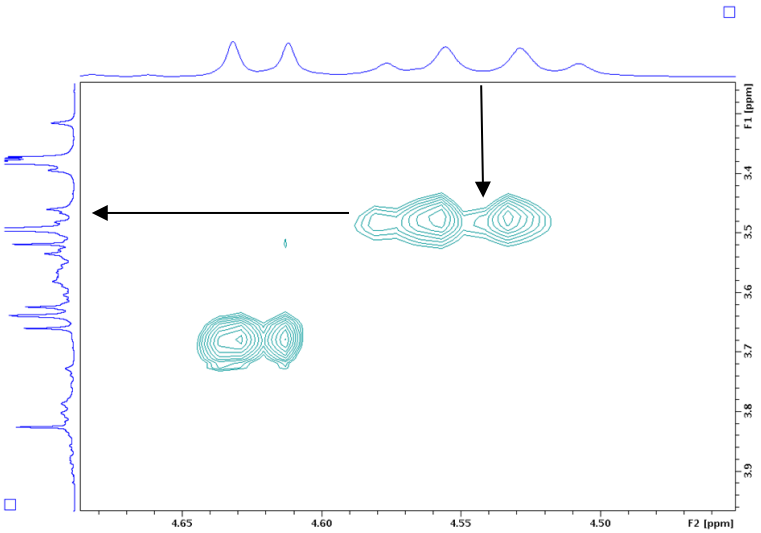
The following partial contour plot of COSY showed a correlation between this last signal and another one at 5.02 ppm (H-1). The signal at 3.46 ppm corresponded then to a proton H-2.



COSY experiment

The 2-*O* substitution by the imidazole group in compound **3** was then proved by the presence of a HMBC correlation between one of the methylenic protons signal (4.51-

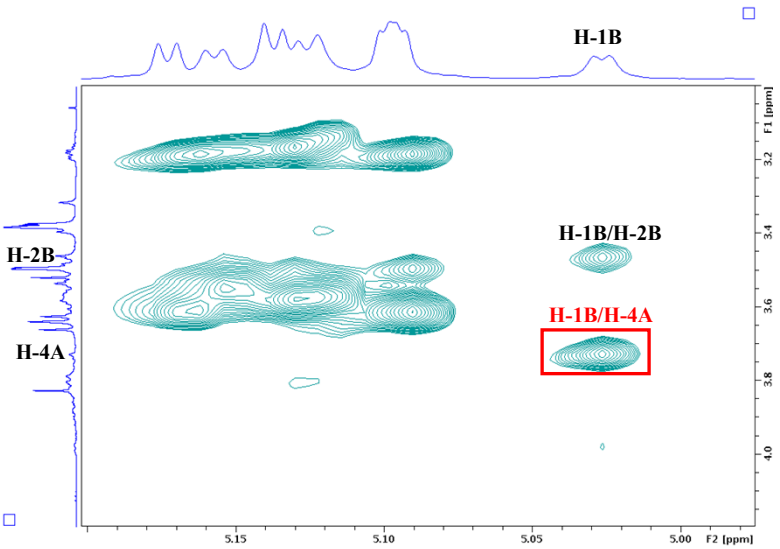
4.56 ppm) and the carbon signal C-2 (79.6 ppm) of the glucose unit B. This result was confirmed by a NOESY experiment that showed a correlation between the respective protons signals at 4.51-4.56 ppm (CH_2-Im) and 3.46 ppm (H-2 of the glucose unit B).



Partial contour plots of the NOESY experiment (area of methylenic protons)

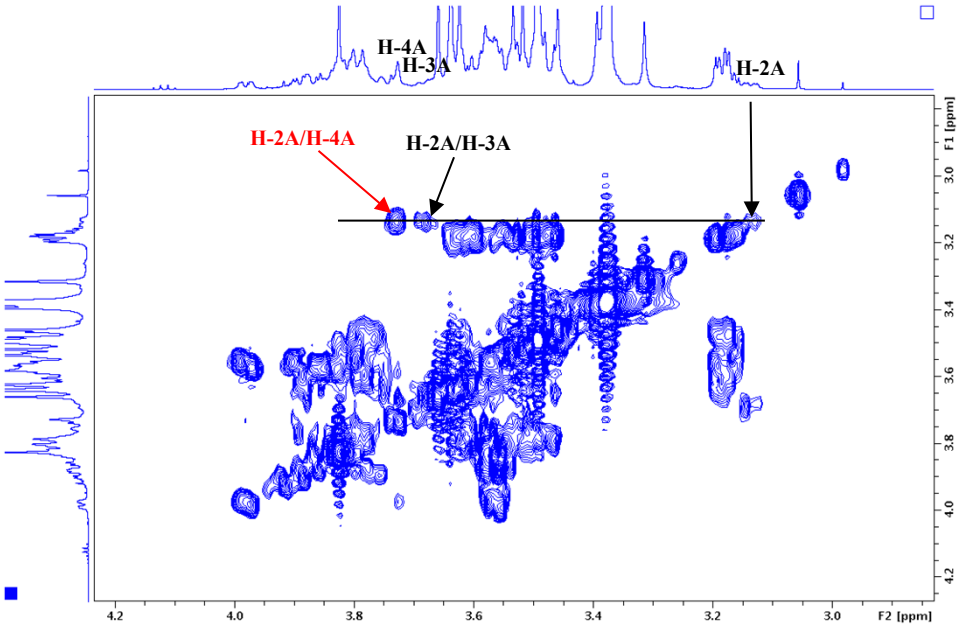
Evidence that the two substituted glucose units are adjacent

The following partial contour plot of COSY showed a correlation between the signals at 5.02 ppm (H-1B) and 3.73 ppm (H-4A).



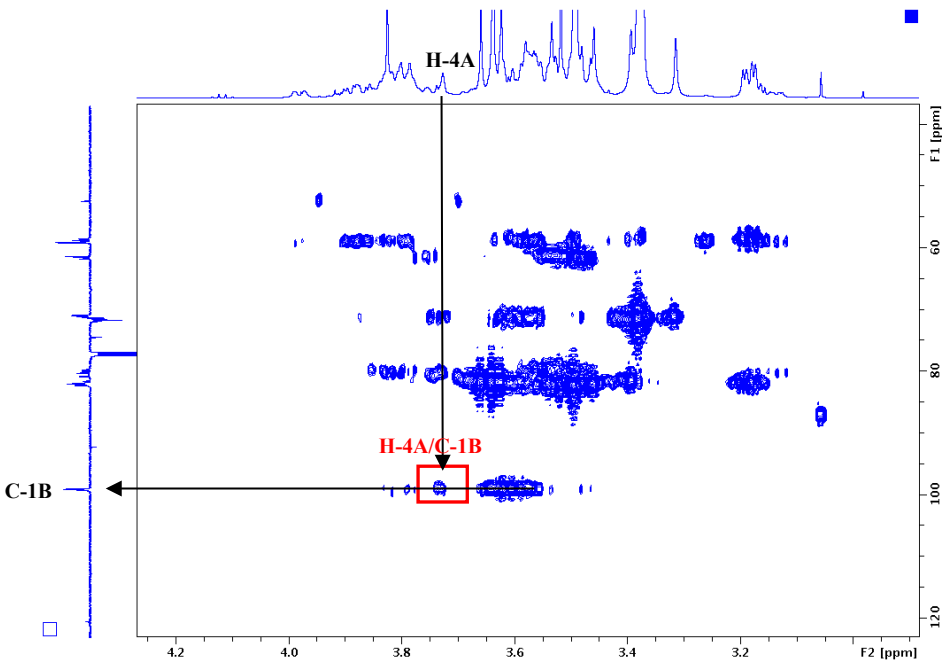
Partial contour plots of the NOESY experiment

The assignment of the H-4A signal was based on the following COSY Relay that showed a correlation between the signals of the protons H-2A/H-3A and H-2A/H-4A.



Partial contour plot of the COSY RELAY experiment

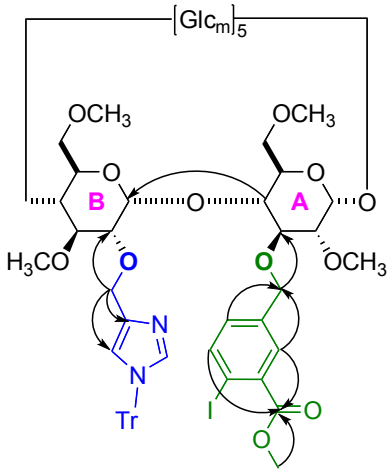
The HMBC experiment proved also that the substituted units are 1,4-linked. It showed a correlation between the signals of H-4A and C-1B.



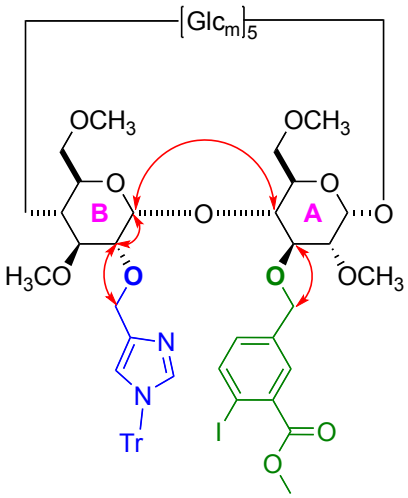
Partial contour plot of HMBC correlation experiment

(F1: DeptQ spectrum, F2: ^{1}H spectrum)

In summary, the main correlations allowing the exact assignments of the substituted positions of compound **3** are undermentioned:



HMBC correlations

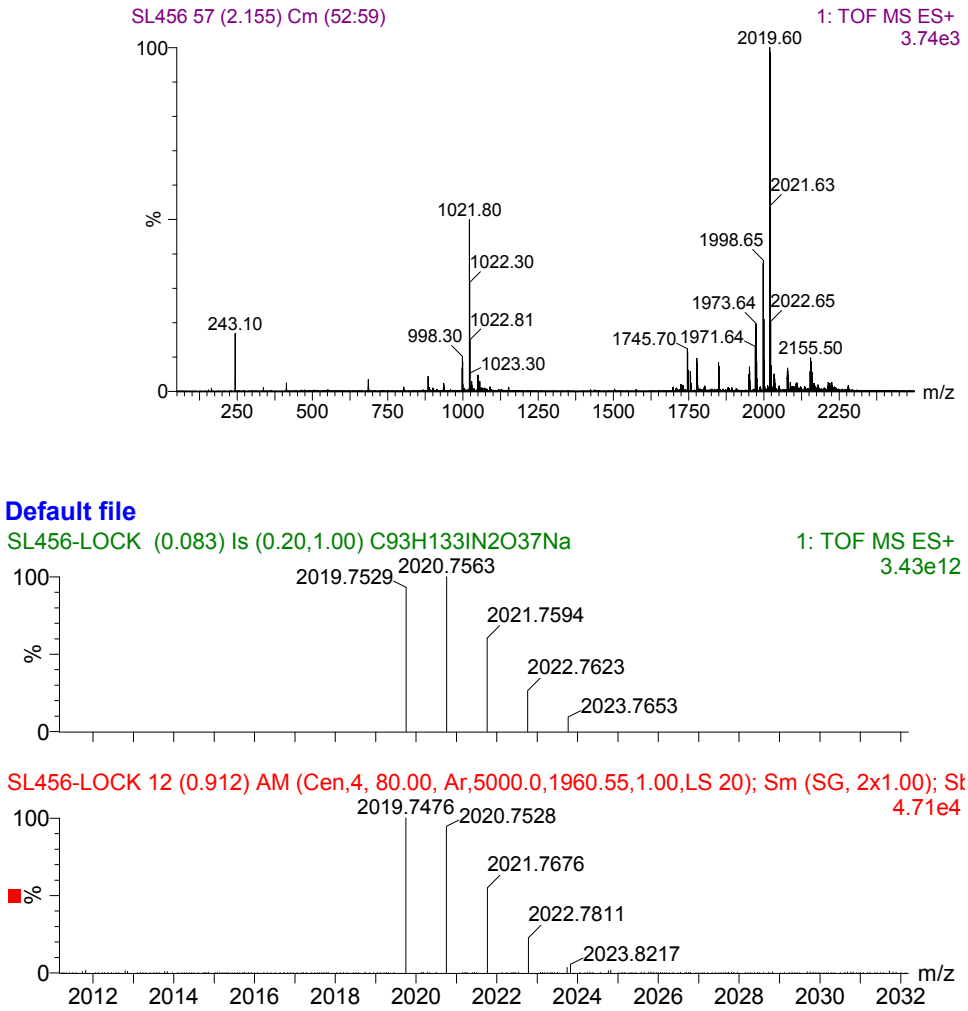


NOESY interactions

4. Routine spectra analysis of compounds 2 and 9b

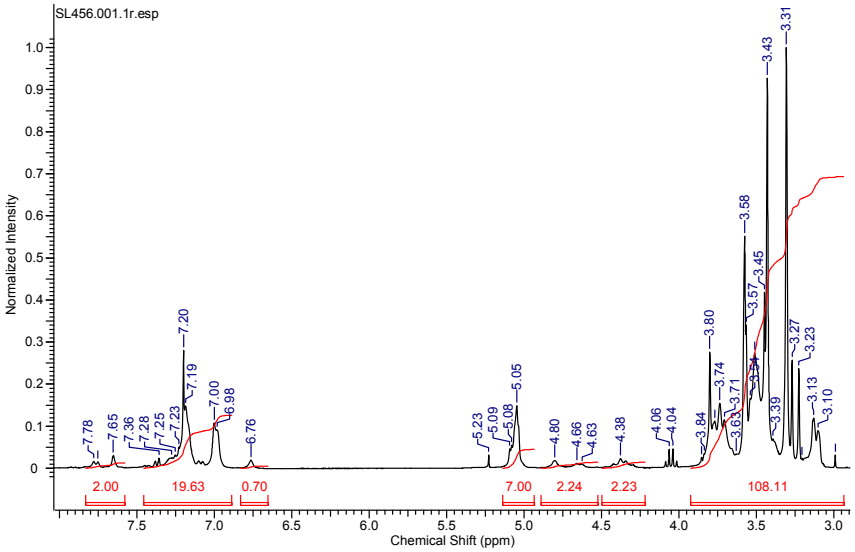
4.1 Compound 2

4.1.1 ESI-HRMS spectrum of 2

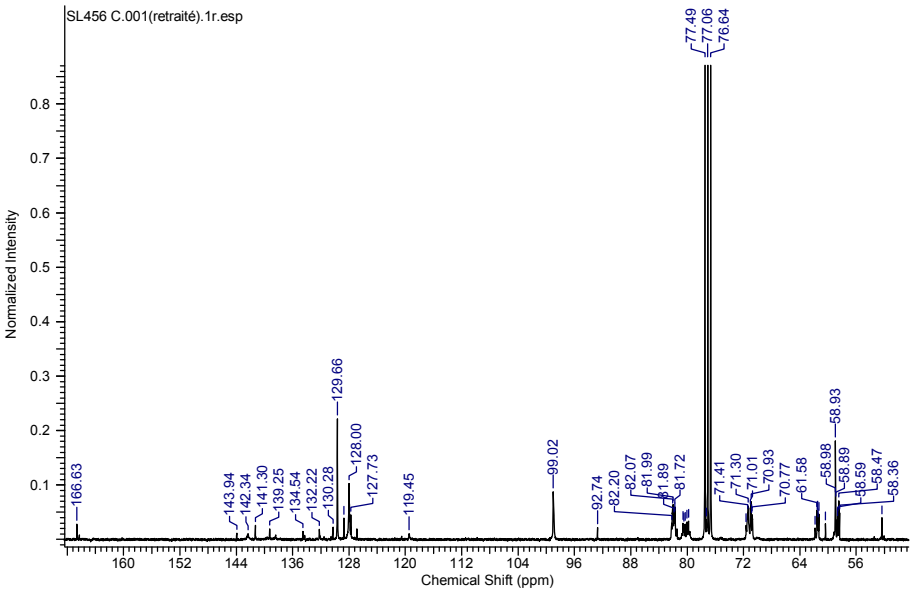


Accurate mass measurement performed on the mono-isotopic ion at m/z 2019.7 revealed only a difference of -2.7ppm (-5.4 mDa) between the measured mass (2019.7476) and the calculated mass (2019.7529) which validates the formula $C_{93}H_{133}N_2O_{37}INa$ corresponding to compound **2**.

4.1.2 ^{1}H NMR spectrum of 2



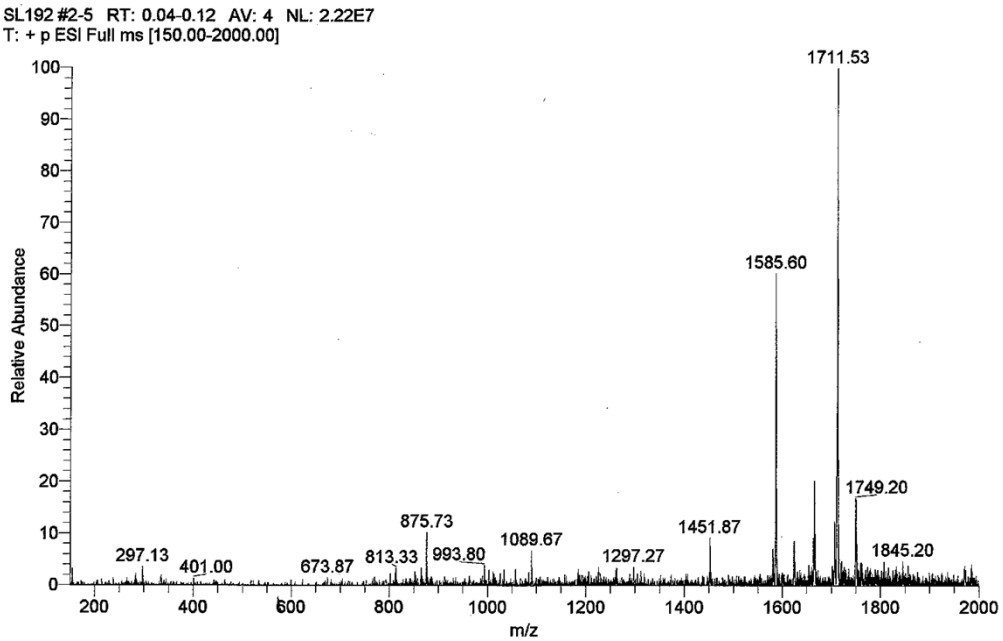
4.1.3 ^{13}C NMR spectrum of 2



5

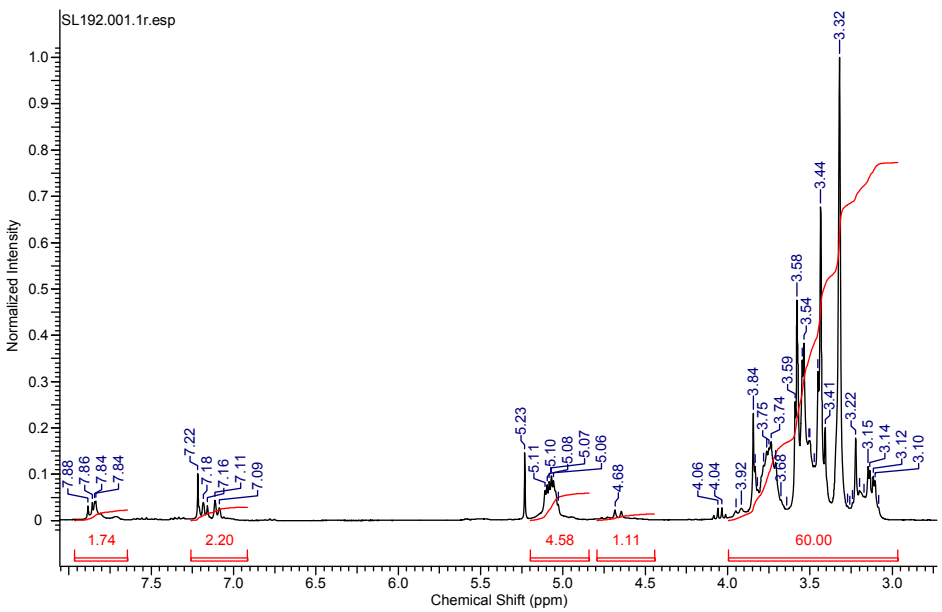
4.2 Compound 9b

4.2.1 ESI-MS spectrum of 9b

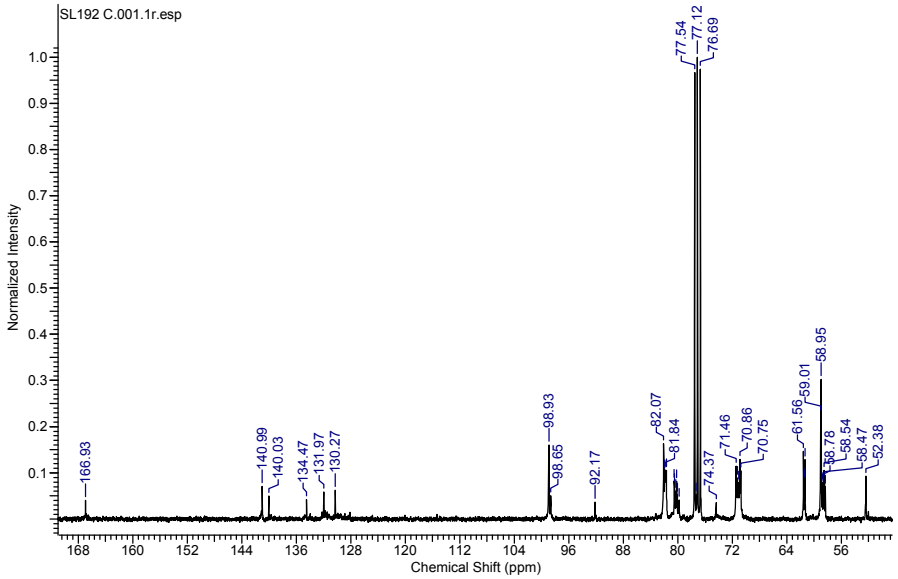


5

^{1}H NMR spectrum of 9b

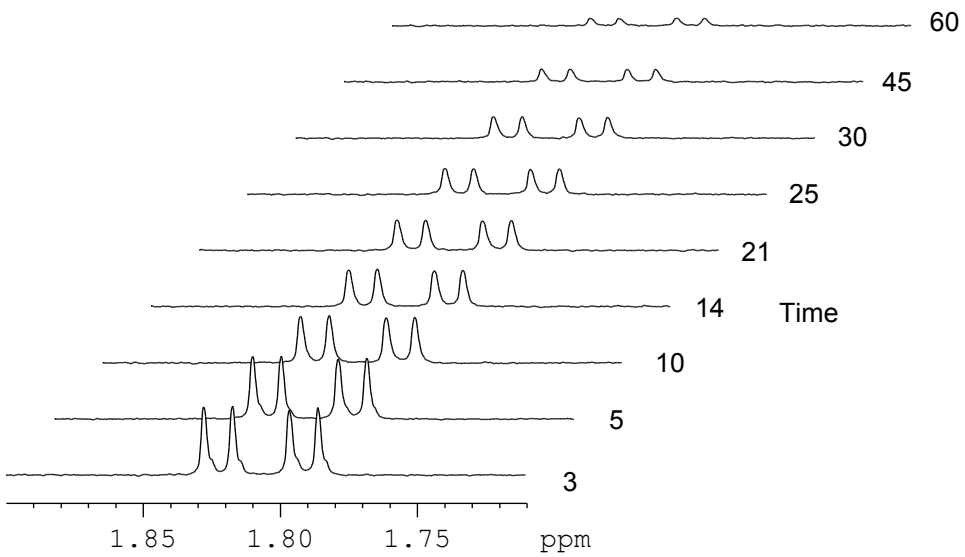


4.2.2 ^{13}C NMR spectrum of 9b



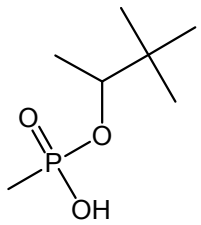
5. Procedure for evaluation of the degradation of soman (GD)

The samples were prepared by adding 450 µL of a solution of 2-iodosobenzoic acid, or compound **4**, **5** or **9** (2 mM in phosphate buffer (0.1 M) at pH 7 and 25°C) and 4.5 µL of a soman solution (8062 µg/mL in CD_3CN) and 50 µL of D_2O. At different time points for up to 1 h 40 min., monitoring was performed by integration of the residual soman signal ($PC\underline{H}_3$, doublet of doublet) between 1.79 and 1.83 ppm.



Staggered view of consecutive 1D ^1H NMR spectra recorded during the degradation of soman by compound 5

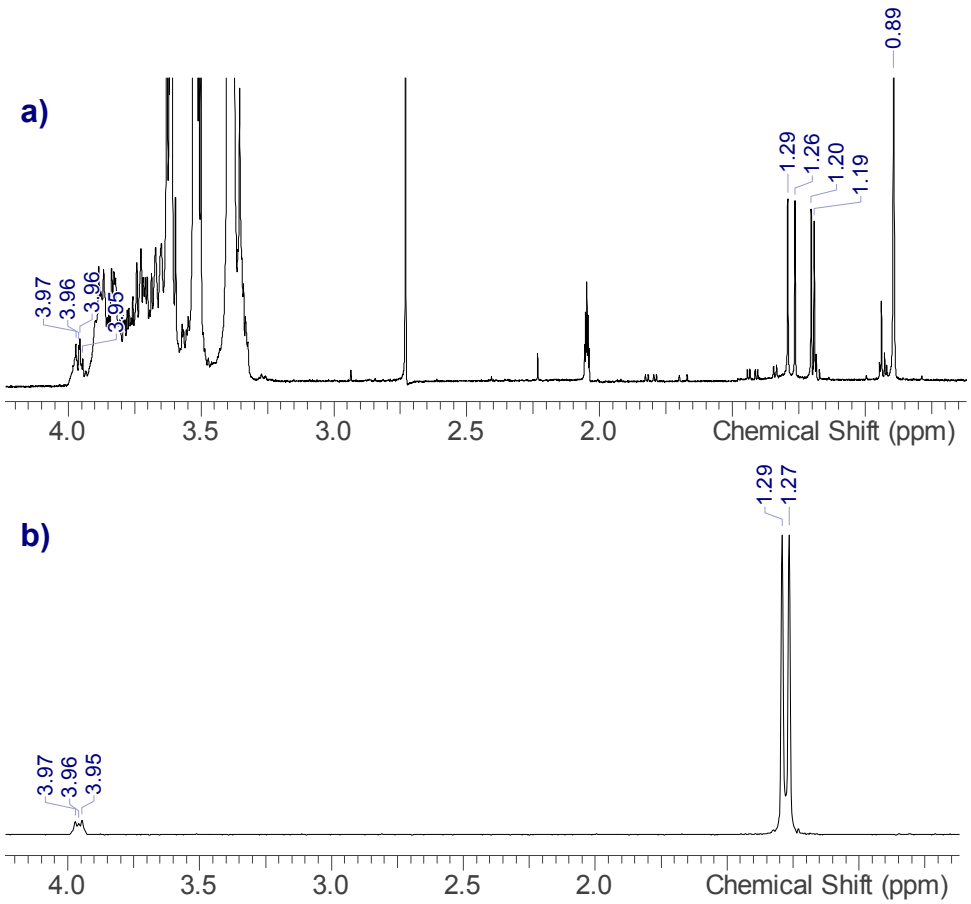
The signal shown (doublet of doublet) correspond to the $PC\underline{H}_3$. For each spectrum, a time acquisition of 1 min 30 sec with ns = 16 was used.



Structure of the degradation product of GD by compound 5

Pinacolic methylphosphonic acid (PMPA, usual name)

3,3-dimethylbutan-2-yl hydrogen methylphosphonate (IUPAC name), 616-52-4 CAS.



Identification of the hydrolysis product (PMPA) on 1D ^{1}H and 2D ^{1}H ^{31}P HMQC NMR spectra

a) 1D ^{1}H NMR with PMPA assignment (spectrum recorded after 1h30 of contact), b) extracted row at 25.5 ppm of the 2D ^{1}H ^{31}P HMQC corresponding to coupled protons with phosphorous atom of PMPA.

Degradation curves of soman (GD)

The degradation curves of soman at 0.4 mM in phosphate buffer (0.1 M) were obtained at pH 7 and 25°C

6. Procedure for evaluation of the acetylcholinesterase inhibitory potential by soman (GD)

GD (1 μM) was incubated with 2-iodosobenzoic acid (500 μM) or cyclodextrin derivatives **4**, **5** or **9** (500 μM) in phosphate buffer (0.1 M) at pH 7 and 37°C for up to 60 min. At different time points (10, 20, 30, 40, 50 and 60 min.), samples were taken and diluted to soman concentration of 500 nM with phosphate buffer. Afterwards, 20 μL of the solution were incubated with 180 μl of AChE (activity was adjusted to 1 U/mL) for 3 min. at 37°C. 50 μL of Ellman solution was then added and the AChE activity was measured spectrophotometrically at 412 nm. Experiments were performed in duplicate and data are means ± SD of 2 experiments.