Supporting information

Synthesis of Fish Antifreeze Neoglycopeptides Using Microwave-Assisted “Click Chemistry”

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GENERAL METHODS

All reagents were purchased as reagent grade and used without further purification. Solvents were dried according to standard methods.¹ Solvents for RP HPLC were purchased as HPLC grade and used without further purification. Fmoc-Ala-Wang resin, Fmoc-Ala, Fmoc-Pro and HBTU were purchased from Advanced ChemTech; Fmoc-Dap was purchased from NeoMPS SA; *i*Pr₂EtNH was purchased from Fluka. Analytical thin layer chromatography was performed using 0.2 mm plates of Kieselgel F254 (Merck) and compounds were visualised by ultraviolet fluorescence or by staining with 4% sulfuric acid in ethanol or sodium permanganate solution, followed by heating the plate as appropriate. Flash chromatography was carried out on Kieselgel F254 S 0.063–0.1 mm (Riedel de Hahn) silica gel with indicated solvents. Microwave-enhanced click reactions were performed in a sealed reaction vessel in a CEM Discover 908010 at 80 °C with IR-monitored temperature control (120 W max.). Infrared spectra were obtained using a Perkin Elmer Spectrum One Fourier Transform infrared spectrometer as a thin film between sodium chloride plates; absorption maxima are expressed in wavenumbers (cm⁻¹). Melting points were determined on an Electrothermal[®] melting point apparatus and are uncorrected. Optical rotations were determined at 20 °C with a Perkin-Elmer 341 polarimeter and are given in units of 10⁻¹ deg cm² g⁻¹. Nuclear magnetic resonance (NMR) spectra were recorded as indicated on either a Bruker AVANCE DRX300 (¹H, 300 MHz, ¹³C, 75 MHz) or on a Bruker AVANCE DRX400 spectrometer (¹H, 400 MHz, ¹³C, 100 MHz). Chemical shifts are reported in parts per million (ppm) relative to the tetramethylsilane signal recorded at δ_{H} 0.00 ppm in CDCl₃-SiMe₄ solvent. The ¹³C values were referenced to the residual chloroform signal at δ_{C} 77.0 ppm in CDCl₃-SiMe₄ solvent. ¹H NMR shift values are reported as chemical shift (δ_{H}), relative integral, multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet; dd, doublet of doublets), coupling constant (*J* in Hz) and assignments. ¹³C values are reported as chemical shift (δ_{C}) and degree of hybridisation. Assignments were made with the aid of DEPT135, COSY, HSQC and HMBC experiments. Low resolution mass spectra were recorded on a VG-70SE mass spectrometer operating at a nominal accelerating voltage of 70 eV (FAB). High resolution mass spectra were recorded using a VG-70SE spectrometer at a nominal resolution of 5000 to 10000 as appropriate. Major and significant fragments are quoted in the form *x* (*y*%), where *x* is the mass to charge

ratio and y is the percentage abundance relative to the base peak. Electrospray ionization mass spectra (ESI-MS) were recorded on a Thermo Finnigan Surveyor MSQ Plus spectrometer. Accurate mass spectra were recorded using Matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDITOF MS) on a Voyager-DE spectrometer using α -cyano-4-hydroxycinnamic acid (CHCA) as matrix. Analytical RP HPLC was performed using a Dionex P680 System or a Dionex Ultimate 3000 System using an analytical column (Phenomenex Gemini C18, 110 Å, 50 mm $\times$ 2.0 mm, 5 μ m or Phenomenex Jupiter C4, 300 Å, 50 mm $\times$ 2.0 mm, 5 μ m), using a linear gradient of 0.1% TFA-H₂O (buffer A) and 0.1% TFA-CH₃CN (buffer B) with detection at 210 nm. Semi-preparative RP HPLC was performed using a Gilson 215 System using a semi-preparative column (Phenomenex Gemini C18, 110 Å, 250 mm $\times$ 10 mm, 5 μ m or Hichrome C18, 150 mm $\times$ 4.6 mm, 5 μ m) at a flow rate of 5 mL min⁻¹, using a linear gradient of 0.1% TFA-H₂O (buffer A) and 0.1% TFA-CH₃CN (buffer B) with detection at 230 nm and 235 nm. Gradient systems were adjusted according to the elution profiles and peak profiles obtained from the analytical RP HPLC chromatograms.

EXPERIMENTAL DATA

Propargyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- α -D-galactopyranoside 1. *N*-acetyl-D-galactosamine (4.42 g, 20 mmol) was suspended in dry DMF (75 mL) and successively treated with sodium hydride (1.04 g, 26 mmol) and propargyl bromide (6.68 mL, 60 mmol). After 2.5 h stirring at room temperature MeOH (2.5 mL) was added and stirring continued for 1 h. MeOH and DMF were distilled and the residue dissolved in Ac₂O (25 mL). At 0 °C NEt₃ (50 mL) was slowly added and the mixture allowed to warm to room temperature. After stirring overnight the mixture was diluted with ethyl acetate (100 mL) and treated with 1 M HCl (80 mL). The aqueous phase was extracted with ethyl acetate (2 × 100 mL) and the organic phase washed with brine (80 mL). The combined organic extracts were dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by flash column chromatography (EtOAc-hexanes 50 : 50 → 75 : 25) afforded 4.41 g (57%) of the *title compound* as a colourless solid: mp 98-100 °C.

R_f 0.23 (80 : 20 EtOAc-hexanes); $[\alpha]_D^{20}$ 47.7 (c 1.1, CHCl₃); **IR (KBr plate neat)** $\nu_{\max}$ 3316, 3266, 2957, 2116, 1737, 1654, 1538, 1369, 1226, 1213, 1117, 1027, 964, 664, 588 cm⁻¹; **¹H NMR (400 MHz, CDCl₃)** δ 6.06 (1 H, d, J = 8.5 Hz, NH), 5.32 (1 H, dd, J = 9.0, 7.4 Hz, 3-CH), 5.20-5.10 (2 H, m, 1-CH and 5-CH), 4.69 (1 H, ddd, J = 8.8, 8.8, 4.6 Hz, 2-CH), 4.06-4.41 (5 H, 4-CH, 6-CH and 1'-CH), 2.46 (1 H, t, J = 2.3 Hz, 3'-CH), 2.14, 2.07, 2.06 (9 H, s, OCOCH₃), 1.99 (3 H, s, NCOCH₃); **¹³C NMR (100 MHz, CDCl₃)** δ 170.6, 170.3, 170.3, 170.0 (CO), 98.4 (CH), 78.4 (C), 78.4 (CH), 75.1 (CH), 74.2 (CH), 70.2 (CH), 62.2 (CH₂), 56.4 (CH), 54.3 (CH₂), 23.1 (CH₃), 20.8 (CH₃), 20.7 (CH₃), 20.6 (CH₃); **m/z (+FAB, *n*-nitrobenzyl alcohol as matrix)** 387 ([M+2]⁺, 6), 386 ([M+1]⁺, 27), 330 (23), 168 (27), 154 (100%); **HRMS (+FAB)** calculated for C₁₇H₂₄NO₉ [MH]⁺: 386.14511, found 386.14462.

Fmoc-L-Ala(N₃)-OH 2. The synthesis was carried in analogy to Lundquist *et al.*² starting from commercial available Fmoc-L-Dap-OH. After purification and lyophilisation the *title compound* was obtained in 88% overall yield as white solid. The ¹H data was in agreement with literature.³

(2S)-2-([(9H-Fluoren-9-ylmethoxy)carbonyl]amino)-3-(4-[(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)oxy]-1H-1,2,3-triazol-1-yl)propanoic acid 3. Propargyl galactopyranoside **1** (58 mg, 0.15 mmol) and Fmoc-L-Ala(N₃)-OH **2** (35 mg, 0.10 mmol) were dissolved in 900 μL of a 1:1:1 mixture of water, *tert*-butyl alcohol and DCM. 10 μL of a stock solution of CuSO₄·5 H₂O (16 mg, 0.10 mmol) and sodium ascorbate (50 mg, 0.25 mmol) in 500 μL water were added and the mixture heated to 80 °C in a sealed reaction vessel in the microwave (120 W max.). After 30 min the mixture was diluted with water (1 mL), acidified with citric acid, followed by extraction with DCM (5 × 5 mL). The combined organic extracts were dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by flash column chromatography (DCM-methanol-acetic acid 98 : 1.7 : 0.3 → 95 : 4 : 1) and lyophilisation afforded 65 mg (88%) of the *title compound* as a colourless solid: mp 95-105 °C.

R_f 0.24 (90 : 9 : 1 DCM–methanol-acetic acid); [α]_D²⁰ 59.1 (*c* 0.9, CHCl₃); **IR (KBr plate neat)** ν_{max} 3345, 2936, 1731, 1528, 1449, 1370, 1220, 1036, 760, 741, 597, 523 cm⁻¹; **¹H NMR (400 MHz, CDCl₃)** δ 7.79-7.72 (2 H, m, Fmoc), 7.63-7.53 (3 H, m, Fmoc and 5''-CH), 7.43-7.25 (5 H, m, Fmoc and OH), 6.69 (1 H, d, *J* = 7.6 Hz, NHCOCH₃), 6.15 (1 H, d, *J* = 6.3 Hz, NHFmoc), 5.36 (1 H, dd, *J* = 8.0, 8.0 Hz, 3-

CH), 5.25-5.16 (1 H, m, 5-CH), 5.08 (1 H, d, $J = 4.4$ Hz, 1-CH), 4.99-4.70 (4 H, m, 2'-CH, 3'-CH and $\text{OCH}_a\text{H}_b\text{C}$), 4.66-4.32 (4 H, m, 2-CH, $\text{OCH}_a\text{H}_b\text{C}$ and CH_2Fmoc), 4.30-4.17 (3 H, m, 4-CH, 6- CH_aH_b and CHFmoc), 4.16-4.01 (1 H, m, 6- CH_aH_b), 2.05, 2.03, 2.02 (9 H, s, OCOCH_3), 1.90 (3 H, s, NCOCH_3); ^{13}C NMR (100 MHz, CDCl_3) δ 171.9 (CO), 170.8 (CO), 170.6 (CO), 170.4 (CO), 170.2 (CO), 155.9 (CO), 143.6 (C), 143.5 (C), 143.1 (C), 141.3 (C), 127.8 (CH), 127.1 (CH), 125.0 (CH), 124.4 (CH), 120.0 (CH), 99.6 (CH), 78.3 (CH), 74.4 (CH), 71.0 (CH), 67.1 (CH_2), 62.2 (CH_2), 60.0 (CH_2), 57.2 (CH), 54.1 (CH), 51.2 (CH_2), 47.1 (CH), 22.6 (CH_3), 20.8 (CH_3), 20.7 (CH_3), 20.6 (CH_3); m/z (+FAB, *n*-nitrobenzyl alcohol as matrix) 738 ($[\text{M}+1]^+$, 4), 178 (5), 154 (100%); HRMS (+FAB) calculated for $\text{C}_{35}\text{H}_{40}\text{N}_5\text{O}_{13}$ $[\text{MH}]^+$: 738.26226, found 738.26209.

General methods for the synthesis of glycopeptides⁴

Glycopeptides were assembled either manually or with the use of a CEM Liberty microwave peptide synthesiser as indicated. Pre-loaded Fmoc-Ala-Wang resin (0.74 mmol g^{-1}) was shaken in DMF for 30 min prior to the synthesis, followed by filtration.

Glycopeptides were assembled manually using Fmoc solid phase peptide synthesis (SPPS) on 0.1 mmol scale using a fritted glass reaction vessel. N^{α} -Fmoc protecting group was deprotected with 20% piperidine solution in DMF (1 $\times$ 5 min, 1 $\times$ 20 min). Coupling of Fmoc-Ala and Fmoc-Pro were performed using method 1, coupling of **3** was performed using method 2.

Glycopeptides were assembled by automated Fmoc-SPPS on a CEM Liberty microwave peptide synthesiser on 0.1 mmol scale using coupling method 3 for Fmoc-Ala and Fmoc-Pro, coupling of **3** was performed using coupling method 4. N^{α} -Fmoc protecting group was deprotected with 20% piperidine solution in DMF (1 $\times$ 0.5 min, 1 $\times$ 3 min).⁵ Fully automated Fmoc-SPPS using building block **2** was performed according to method 3.

Cleavage of the resin was performed by treatment with 95%:2.5%:2.5% (v/v/v) trifluoroacetic acid:*i*Pr₃SiH:water for 2 h at room temperature. The resin was filtered, and washed with trifluoroacetic acid (2 $\times$) and CH_2Cl_2 (3 $\times$). The filtrate was collected and the solvent was removed *in vacuo*. Glycopeptides were precipitated from cold Et_2O , centrifuged (10 min), Et_2O was decanted, glycopeptides were suspended in

fresh Et₂O and centrifuged again. Crude products were lyophilised from CH₃CN/water + 0.1% v/v trifluoroacetic acid and depending on the purity purified by RP-HPLC or the next reaction step was undertaken.

Coupling method 1 (manual Fmoc-SPPS, Fmoc-AA)

N^α-Protected amino acid (2.5 eq.) was dissolved in DMF, HBTU (2.45 eq.) was then added and shaken until dissolved. The solution was transferred to the reaction vessel and shaken for 2 min, followed by the addition of *i*Pr₂EtN (5 eq.). The mixture was shaken for 45 min, filtered, washed with DMF (3 ×), ethanol (3 ×) and CH₂Cl₂ (3 ×).

Coupling method 2 (manual Fmoc-SPPS, building block 3)

3 (1.5 eq.) was dissolved in DMF, HBTU (1.45 eq.) was then added and shaken until dissolved. The solution was transferred to the reaction vessel and shaken for 2 min, followed by the addition of *i*Pr₂EtN (5 eq.). The mixture was shaken for 45 min, filtered, washed with DMF (3 ×), ethanol (3 ×) and CH₂Cl₂ (3 ×).

Coupling method 3 (automated Fmoc-SPPS, Fmoc-AA)

Couplings of Fmoc-Ala, Fmoc-Pro and **2** (5 eq.) were carried out in 15 min at 80 °C (20 W) using a CEM Liberty microwave peptide synthesiser in presence of HBTU (4.5 eq.) and *i*Pr₂EtN (10 eq.) in DMF.

Coupling method 4 (automated Fmoc-SPPS, building block 3)

3 (1.5 eq.) was dissolved in DMF, HBTU (1.45 eq.) was then added and shaken until dissolved. The solution was transferred to the sealed microwave reactor, DMF added and the reaction mixture was microwaved for 15 min at 80 °C (20 W).

H₂N-Ala-Ala-X₁-Ala-Ala-X₁-Pro-Ala-OH 4a (X₁: [3-(4-{[(2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranosyl)oxy]-1*H*-1,2,3-triazol-1-yl})]-L-alanine).

Route A: Glycopeptide **4a** was assembled manually using Fmoc-SPPS according to coupling methods 1 and 2. Purification by RP HPLC on a Phenomenex Gemini C18 column at a flow rate of 5 mL min⁻¹ using a linear gradient of 1% B to 41% B over 20 min, followed by lyophilisation yielded glycopeptide **4a** as a colourless amorphous solid in *ca.* 95% purity according to analytical HPLC with an overall yield of 10%; Rt 18.0 min (Dionex Gemini C18, 1% to 41% B over 20 min, 0.2 mL min⁻¹); *m/z* (ESI-MS) 733.05 ($[M+2]/2$ requires 733.2).

Route B: Propargyl galactopyranoside **1** (15.1 mg, 0.039 mmol) and crude peptide **5a** (9.1 mg, 0.013 mmol) were dissolved in 300 μ L of a 1:1 mixture of water and *tert*-butyl alcohol. 10 μ L of a stock solution of CuSO₄·5 H₂O (25 mg, 0.16 mmol) and sodium ascorbate (78 mg, 0.39 mmol) in 600 μ L water were added and the mixture heated to 80 °C in a sealed reaction vessel in the microwave (120 W max.). After 10 min the reaction was quenched by addition of dry ice and the solvent removed *in vacuo* to afford crude **4a**. Purification was performed as described above to yield **4a** in 19%.

H₂N-Ala-(Ala-X₁-Ala-Ala-X₁-Pro)₂-Ala-OH 4b (X₁: [3-(4-{[(2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranosyl)oxy]-1*H*-1,2,3-triazol-1-yl})]-L-alanine).

Route A: Glycopeptide **4b** was assembled using automated Fmoc-SPPS according to coupling methods 1 and 2. Purification by RP HPLC on a Phenomenex Gemini C18

column at a flow rate of 5 mL min⁻¹ using a linear gradient of 1% B to 61% B over 30 min followed by lyophilisation yielded glycopeptide **4b** as a colourless amorphous solid in *ca.* 81% purity according to analytical HPLC with an overall yield of 6%; *Rt* 13.7 min (Dionex Gemini C18, 1% to 91% B over 20 min, 0.2 mL min⁻¹); *m/z* (ESI-MS) 1386.7 ($[M+2]/2$ requires 1386.3).

Route B: Propargyl galactopyranoside **1** (20.8 mg, 0.054 mmol) and peptide **5b** (11.1 mg, 0.009 mmol) were dissolved in 300 μ L of a 1:1 mixture of water and *tert*-butyl alcohol. 20 μ L of a stock solution of CuSO₄·5 H₂O (34 mg, 0.22 mmol) and sodium ascorbate (106 mg, 0.54 mmol) in 600 μ L water were added and the mixture heated to 80 °C in a sealed reaction vessel in the microwave (120 W max.). After 10 min the reaction was quenched by addition of dry ice and the solvent removed *in vacuo* to afford crude **4b**. Purification was performed as described above to yield **4b** in 72% yield.

H₂N-Ala-Ala-X₂-Ala-Ala-X₂-Pro-Ala-OH 5a (X₂: azido-L-alanine). Glycopeptide **5a** was assembled using fully automated Fmoc-SPPS according to coupling method 3 in *ca.* 76% purity (28% yield) according to analytical HPLC. *Rt* 9.4 min (Dionex Gemini C18, 1% to 91% B over 20 min, 0.2 mL min⁻¹); *m/z* (ESI-MS) 695.1 ($[M+H]^+$ requires 695.3).

H₂N-Ala-(Ala-X₂-Ala-Ala-X₂-Pro)₂-Ala-OH 5b (X₂: azido-L-alanine). Glycopeptide **5b** was assembled using fully automated Fmoc-SPPS according to coupling method 3. Purification by RP HPLC on a Phenomenex Gemini C18 column at a flow rate of 5

mL min⁻¹ using a linear gradient of 1% B to 41% B over 20 min followed by lyophilisation yielded glycopeptide **5b** as a colourless amorphous solid in *ca.* 99% purity according to analytical HPLC and with an overall yield of 9%; *Rt* 11.3 min (Dionex Gemini C18, 1% to 91% B over 20 min, 0.2 mL min⁻¹); *m/z* (ESI-MS) 1230.1 ($[M+H]^+$ requires 1229.6).

H₂N-Ala-Ala-X₃-Ala-Ala-X₃-Pro-Ala-OH **6a** (X₃: [3-(4-[(α-D-galactopyranosyl)oxy]-1*H*-1,2,3-triazol-1-yl}))-L-alanine). Glycopeptide **4a** (2.8 mg, 1.9 μmol) was dissolved in MeOH and the pH value adjusted to 11.3 with 1 M NaOMe. After 2 h stirring at room temperature, dry ice was added and the crude mixture lyophilised from CH₃CN/water + 0.1% v/v trifluoroacetic acid. Purification by RP HPLC on a Phenomenex Gemini C18 column at a flow rate of 5 mL min⁻¹ using a linear gradient of 1% B to 18% B over 15 min followed by lyophilisation yielded **6a** (1.0 mg, 43%) as a colourless amorphous solid in *ca.* 93% purity according to analytical HPLC; *Rt* 10.6 min (Dionex Gemini C18, 1% to 30% B over 15 min, 0.2 mL min⁻¹); *m/z* (ESI-MS) 1214.1 ($[M+H]^+$ requires 1213.5).

H₂N-Ala-(Ala-X₃-Ala-Ala-X₃-Pro)₂-Ala-OH **6b** (X₃: [3-(4-{[(α-D-galactopyranosyl)oxy]-1*H*-1,2,3-triazol-1-yl})]-L-alanine). Glycopeptide **4b** (5.5 mg, 1.9 μmol) was dissolved in MeOH and the pH value adjusted to 11.3 with 1 M NaOMe. After 1 h stirring at room temperature, dry ice was added and the crude mixture lyophilised from CH₃CN/water + 0.1% v/v trifluoroacetic acid. Purification by RP HPLC on a Hichrome C18 column at a flow rate of 5 mL min⁻¹ using a linear gradient of 1% B to 18% B over 20 min followed by lyophilisation yielded **6b** (1.5 mg, 34% yield) as a colourless amorphous solid in *ca.* 97% purity according to analytical HPLC; Rt 8.4 min (Phenomenex Jupiter C4, 1% to 51% B over 20 min, 0.5 mL min⁻¹); *m/z* (ESI-MS) 2267.0 ([*M*+2*H*]⁺ requires 2267.0), 1133.5 ([*M*+2]/2 requires 1133.5).

REFERENCES

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- (3) Oh, K. I.; Lee, J. H.; Joo, C.; Han, H.; Cho, M., beta-Azidoalanine as an IR probe: Application to amyloid A beta(16-22) aggregation. *J. Phys. Chem. B* **2008**, 112, (33), 10352-10357.
- (4) Chan, W. C.; White, P. D, *Fmoc Solid Phase Peptide Synthesis: a Practical Approach*, Oxford University Press, 2000.
- (5) Conditions recommended by CEM Microwave Technology

^1H AND ^{13}C NMR OF

Compound 1

nm175_1
 NAME nm175_1
 EXPNO 10
 PROCNO 1
 Date_ 20090115
 Time 18.16
 INSTRUM spect
 PROBHD 5 mm BBO BB-1H
 PULPROG zgpg30
 TD 32768
 SOLVENT CDCl3
 NS 32
 DS 0
 SWH 8169.935 Hz
 FIDRES 0.249327 Hz
 AQ 2.0054517 sec
 RG 101.6
 DW 61.200 usec
 DE 6.50 usec
 TE 300.0 K
 D1 0.1000000 sec
 D11 1
 D10 1
 ===== CHANNEL f1 =====
 NUC1 ^1H
 P1 18.70 usec
 PL1 0.50 dB
 SFO1 400.1321007 MHz
 SI 16384
 SF 400.1300610 MHz
 WILW EM
 SSB 0
 LB 0.10 Hz
 GB 0
 PC 0.80

NM175

NM175

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NAME          nm175_1
EXPNO         11
PROCNO        1
Date_         20090115
Time          18.52
INSTRUM       spect
PROBHD        5 mm BBO BB-1H
PULPROG       zgpg30
TD            65536
FIDRES        0.326
SOLVENT       CDCl3
NS            400
DS            0
SWH           24038.461 Hz
AQ            0.16788 Hz
RG            1.363188 sec
RC            6502
DW            20.800 usec
DE            6.50 usec
TE            300.0 K
D1            0.63167691 sec
d11           0.03000000 sec
DELTA         0.53167669 sec
TD0           1
===== CHANNEL f1 =====
NUC1          13C
P1            9.00 usec
PL1           -1.50 dB
SFO1          100.6238978 MHz
===== CHANNEL f2 =====
CPDPRG2       waltz16
NUC2          1H
PCPD2         100.00 usec
PL2           0.50 dB
PL12          15.06 dB
PL13          18.80 dB
SFO2          400.146006 MHz
SF            32768
WDW           EM
SSB           0
LB            2.00 Hz
GB            0
PC            0.80

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Compound 3

NM187

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NAME          nm187-1
EXPNO         10
PROCNO        1
Date_         20090115
Time          17.09
PULPROG       zgpg30
PCPDPRG       zgpg30
SOLVENT       CDCl3
NS            32
DS            0
SWH           8169.935 Hz
FIDRES        0.249327 Hz
AQ            2.0054517 sec
RG            101.6
DW            61.200 usec
DE            8.000 usec
TE            300.2 K
D1            0.1000000 sec
D2            1
TD0           1
===== CHANNEL f1 =====
NUC1          1H
P1            18.70 usec
PL1           0.50 dB
SFO1          400.1321007 MHz
SI            16384
SF            400.1300102 MHz
WDW           EM
SSB           0
LB            0.10 Hz
GB            0
PC            0.80

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NM187

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NAME          nm187_1
EXPNO         11
PROCNO        1
Date_         20090115
Time_         17.35
INSTRUM       spect
PROBHD        5 mm BBO BB-1H
PULPROG       zgpg30
TD            65536
SOLVENT       CDCl3
NS            400
DS            0
SWH           24038.461 Hz
FIDRES        0.366788 Hz
AQ            1.3631988 sec
RG            6502
DM            20.800 usec
DE            6.50 usec
TE            300.2 K
D1            0.6316000 sec
d11           0.03000000 sec
DELTA         0.53167689 sec
TD0           1
===== CHANNEL f1 =====
NUC1          13C
P1            13C
PL1           9.00 usec
PL12          -1.00 dB
SFO1         100.6236978 MHz
===== CHANNEL f2 =====
CEPRPG2       waltz16
NUC2          1H
P2            100.00 usec
PL2           0.00 dB
PL12          18.30 dB
PL13          18.30 dB
SFO2         400.1318006 MHz
SI            32768
SF           100.6127780 MHz
WDW           EM
SSB           0
GB            0
PC            0.80

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LC-MS of compound **4a**

HPLC and MS of compound **4a** (crude, ca. 71% purity);
 Dionex Gemini C18, 110Å, 50 mm × 2.0 mm, 5 μm,
 1% to 41% B over 20 min, 0.2 mL min⁻¹

*HPLC and MS of compound **4a** (purified, ca. 95% purity);*
Dionex Gemini C18, 110Å, 50 mm × 2.0 mm, 5 μm,
1% to 41% B over 20 min, 0.2 mL min⁻¹

LC-MS of compound **4b**

*HPLC and MS of compound **4b** (crude, ca. 39% purity);*
 Dionex Gemini C18, 110Å, 50 mm × 2.0 mm, 5 μm,
 1% to 91% B over 20 min, 0.2 mL min⁻¹

LC-MS of compound 4b (purified, *ca.* 81% purity);
Dionex Gemini C18, 110Å, 50 mm × 2.0 mm, 5 μm,
1% to 91% B over 20 min, 0.2 mL min⁻¹

LC-MS of compound **5a**

HPLC and MS of compound **5a** (crude, *ca.* 76% purity);
 Dionex Gemini C18, 110Å, 50 mm × 2.0 mm, 5 μm ,
 1% to 91% B over 20 min, 0.2 mL min⁻¹

LC-MS of compound **5b**

5b

HPLC and MS of compound **5b** (crude, ca. 56% purity);
Dionex Gemini C18, 110Å, 50 mm × 2.0 mm, 5 μm,
1% to 91% B over 20 min, 0.2 mL min⁻¹

LC-MS of compound **5b** (purified, ca. 99% purity);
Dionex Gemini C18, 110Å, 50 mm × 2.0 mm, 5 μm,
1% to 91% B over 20 min, 0.2 mL min⁻¹

LC-MS of compound **6a**

HPLC and MS of compound **6a** (purified, ca. 93% purity);
 Dionex Gemini C18, 110Å, 50 mm × 2.0 mm, 5 µm,
 1% to 30% B over 15 min, 0.2 mL min⁻¹

LC-MS of compound **6b**

*HPLC and MS of compound **6b** (purified, ca. 97% purity);*
Phenomenex Jupiter C4, 150 mm × 4.6 mm, 5 μm,
1% to 51% B over 20 min, 0.5 mL min⁻¹