Supporting Information

RP-HPLC traces for scaffolds and peptide ligands at 220 (black) and 280 (red) nm:



gradient: 9→45% acetonitrile in 0.1% TFA over 30 minutes at 1 mL/min





gradient: 9→72% acetonitrile in 0.1% TFA over 30 minutes at 1 mL/min.



gradient: 9→45% acetonitrile in 0.1% TFA over 30 minutes at 1 mL/min





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gradient: 9→45% acetonitrile in 0.1% TFA over 30 minutes at 1 mL/min

N₃ ↔ NH-LLADTTHHRPWTG-OH



gradient: 9→45% acetonitrile in 0.1% TFA over 30 minutes at 1 mL/min



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gradient: 9→72% acetonitrile in 0.1% TFA over 30 minutes at 1 mL/min.

RP-HPLC traces for the macrocyclization of NH_2 -Pra-Lys(Boc)-Pra-Pro-Gly-Pra-Ala-Pra-Pro-Gly-OH to *cyclo*-(Pra-Lys(Boc)-Pra-Pro-Gly-Pra-Ala-Pra-Pro-Gly) using HBTU or at 220 (black) and 280 (red) nm:

HBTU activation:



Reference: NH₂-Pra-Lys(Boc)-Pra-Pro-Gly-Pra-Ala-Pra-Pro-Gly-OH



gradient: 9->45% acetonitrile in 0.1% TFA over 30 minutes at 1 mL/min

Reaction mixture:



R_t= 26 min: guanidinated open chain precursor

R_i= 30 min: cyclo-(Pra-Lys(Boc)-Pra-Pro-Gly-Pra-Ala-Pra-Pro-Gly)

gradient: 9→45% acetonitrile in 0.1% TFA over 30 minutes at 1 mL/min

PyBOP activation:

NH₂-Pra-Lys(Boc)-Pra-Pro-Gly-Pra-Ala-Pra-Pro-Gly-OH

Pra Gly Pra Lys BocHN Ho Gly Pra Gly BocHN

Reference: NH₂-Pra-Lys(Boc)-Pra-Pro-Gly-Pra-Ala-Pra-Pro-Gly-OH



PyBOP / DIEA

gradient: 30→80% methanol in 0.1% TFA over 30 minutes at 1 mL/min

Reaction mixture:



R_i= 24 min: cyclo-(Pra-Lys(Boc)-Pra-Pro-Gly-Pra-Ala-Pra-Pro-Gly)

gradient: 30→80% methanol in 0.1% TFA over 30 minutes at 1 mL/min

ESI-MS:



^{m/z} Conjugate 8: C₅₆H₇₈N₂₂O₁₈ Calc. Mass: 1346.6



Conjugate 9: C140H194N50O46, Calc. Mass: 3311.4



Conjugate 10: C216H326N78O62, Calc. Mass: 5004.5





Conjugate 11: C₂₅₆H₄₀₂N₇₄O₁₀₂, Calc. Mass: 6144.9

Conjugate 12: C324H474N106O90, Calc. Mass: 7289.6

Conjugate 13: C₂₄₀H₃₄₄N₈₀O₆₇S, Calc. Mass: 5450.6

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GFC traces at 220 nm: (eluent: 150 m aq. NaCl at flow rate 0.75 mL / min)

Conjugate 9:

Conjugate 10:

Peptide ligand 5:

30.0 min

SSP Geminyx Version 1.91 INTEGRATION SYS1 - MDGFC3.SMP: (modified) No. 23: mdgfc3 23.01 Channel 1: DAD 1 chrom. 220/ 5 Acquired : 10.12.08 Processed: 22.12.08 09:44:56 11:16 No Text 20 40 60 80 100 120 150 -10 0.3 mAbs 3.0 6.0 9.0-12.0-15.0 - 14.99:1 18.0-21.0 24.0-27.0-

25

Conjugation product 10:

Conjugate 11:

Peptide ligand 6:

Conjugation product 11:

Conjugate 12:

Peptide ligand 7:

SSP Geminyx Version 1.91 INTEGRATION SYS1 - MDGFC3.SMP: (modified) No. 25: mdgfc3 25.01 Channel 1: DAD 1 chrom. 220/ 5 Acquired : 10.12.08 10:48:16 Processed: 22.12.08 11:26 No Text 150 225 300 375 500 -30 75 0.3 mAbs 3.0 6.0 9.0-12.0-13.68:1 15.0 15.29:2 16.49:3 18.0-21.0 24.0-27.0-30.0 min

Conjugation product 12:

Conjugate 13:

Peptide ligand 5 see "conjugate 10"

Conjugation product 13:

GFC trace of 13 at 220 nm and 485 nm:

