Synthesis of structurally diverse and defined bivalent mannoses on saccharide scaffolding

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Analytical data for compounds 2-5

Compound 2

¹H-NMR (500 MHz, D₂O): δ 8.02 (s, 4H, aromatic H), 5.40 (apparent s, 2H, mannose H-1), 5.35 (d, 2H, $J_{1,2}$ 9.3, glucuronic acid H-1), 5.30 (t, 2H, $J_{3,4}$ 10.0, $J_{4,5}$ 10.0, mannose H-4), 4.18 (apparent s, 2H, mannose H-2), 4.08 (m, 2H, mannose H-5), 3.99 (d, 2H, $J_{4,5}$ 10.0, H-5), 3.97 (m, 2H, mannose H-3', partially overlapping with H-5), 3.95-3.78 (m, 8H, mannose H-6a, H-6b, and glucuronic acid H-4 and H-3), 3.76 (t, 2H, $J_{2,3}$ 9.0, glucuronic acid H-2).

¹³C-NMR (D₂O): δ 173.6 (2s, CONH and COOH), 139.4 (s, aromatic C), 130.7 (d, aromatic CH), 103.7 (d, mannose C-1), 85.1 (d, glucuronic acid C-1), 82.6, 75.6, 75.2, 73.3, 73.2, 73.1 (2s), 69.4 (each d), 63.6 (t).

ES-HRMS: Found 839.2230, required 839.2206 [M-H]⁻.

Compound 3

¹H-NMR (D₂O, 300MHz) δ (ppm): 7.96 (4H, s, aromatic H), 5.43 (2H, d, $J_{1,2}$ = 8.4 Hz, glucose H-1), 4.90 (2H, s, mannose H-1), 3.98-3.55 (24H, m).

¹³C-NMR (CDCl₃, 75MHz) δ (ppm): 173.7 (NHCO), 139.4 (s, aromatic C), 130.8 (d, aromatic CH) 102.5 (d, mannose C-1), 83.0 (d, glucose C-1), 79.6, 78.8, 75.5, 74.5, 73.4, 72.7, 72.1, 69.4 (each d), 68.5 and 63.7 (each t).

ES-HRMS: [M+Na⁺] calcd. 835.2596, found 835.2571.

Compound 4

¹H-NMR (D₂O, 300MHz) δ (ppm): 7.96 (4H, s, aromatic H), 5.36 (2H, s, mannose H-1), 5.22 (2H, d, $J_{1,2}$ = 9.0Hz, glucose H₁), 4.10 (2H, s), 3.95-3.59 (22H, m), 3.60 (2H, t, $J_{1,2}$ = 9.0 Hz, $J_{2,3}$ = 9.0 Hz, glucose H₂).

¹³C-NMR (D₂O, 75MHz) δ (ppm): 173.7 (NHCO), 139.4 (s, aromatic C), 130.8 (d, aromatic CH) 104.2 (d, C-1, mannose), 82.6 (d, C-1, glucose), 79.9, 79.3, 78.5, 76.6, 74.7, 73.2, 73.1, 69.4 (each d), 63.7, 63.4 (each t).

ES-HRMS: (M-H)⁻ calcd. 811.2620, found 811.2656.

Compound 5

¹H-NMR (500 MHz, D₂O): δ 7.60 (s, 4H, aromatic H), 5.35 (d, 2H, $J_{1,2}$ 1.4, mannose H-1), 4.98 (d, 2H, $J_{1,2}$ 9.0, glucuronic acid H-1), 4.24 (d, 2H, $J_{4,5}$ 9.5, glucuronic acid H-5), 4.14 (m, 2H, mannose H-2), 4.06 (m, 2H, mannose H-6), 3.96-3.86 (m, 10H, mannose H-

6a, H-4, H-5, H-3, glucose H-3), 3.81 (t, 2H, $J_{3,4}$ 10.0, glucuronic acid H-4), 3.54 (t, 2H, $J_{2,3}$ 9.0, glucuronic acid H-2).

^{13}C -NMR (D_2O): δ 171.2 (s, CONH), 136.4 (s, aromatic C), 125.9 (d, aromatic CH), 103.8 (d, mannose C-1), 93.1 (d, glucuronic acid C-1), 84.1, 79.9, 75.7, 74.4, 74.0, 73.2, 73.1, 69.3 (each d), 63.5 (t, mannose C-6)

ES-HRMS: Found 835.2626, required 835.2594 $[\text{M}+\text{H}]^+$

^1H -NMR Spectrum of 2

¹³C-NMR of 2

¹H-NMR Spectrum of 3

¹³C-NMR Spectrum of 3

¹H-NMR Spectrum 4

¹³C-NMR Spectrum of 4

¹H-NMR of 5

^{13}C -NMR of 5

-KXN259 UEP
Tosin
lse Sequence: s2pu1
olvent: CDCl3
emp. 30.0 C / 303.1 K
er: 1-14-87
OVA-500 "sunucd500"
play, delay 0.800 sec
ulse, 50.0 degrees
eg, time 1.301 sec
fidh 33641.7 Hz
02048 repetitions
SERVE C13, 125.6793725 MHz
COUPLE H1, 499.8207654 MHz
power 39 dB
continuously on
ALTZ-16 modulated
FA PROCESSING
line broadening 1.0 Hz
size 131072
lat time 58615 hr, 8 min, 32 sec

¹H-NMR of 7

^{13}C -NMR of 7

Archive directory: /export/home/vnmr3/vnmr-sys/data
Sample directory: auto_CustomQ_10c35_2003-01-16
Pulse Sequence: szpu1
Solvent: cdcl3
Temp: 25.0 C / 298.1 K
File: P_Murphy_3533
NOVA-300 "sunucds1"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.199 sec
Width 18850.1 Hz
2000 repetitions
OBSERVE C13, 75.4069355 MHz
DECUPLE H1, 299.8897221 MHz
Power 35 dB
continuously on
WALTZ-16 modulated
MIRA PROCESSING
Line broadening 1.0 Hz
F size 65536
Total time 1 hr, 13 min, 36 sec

¹H-NMR of 9

¹³C-NMR of 9

178 rep III-co1/chrom-carbon
: P. Murphy_21_3
Sequence: s2pul
t: cdc13
25.0 C / 298.1 K
#21
- delay 1.000 sec
45.0 degrees
t: 45.0 sec
1850.1 Hz
repetitions
E C13: 75.4069355 MHz
LE H1: 299.8097221 MHz
35 dB
nuously on
#6 modulated
#00333ND
broadcasting 1.0 Hz
65536
time 1 hr, 13 min

¹³C-NMR of 11

¹³C-NMR of 12

¹H-NMR of 14

¹³C-NMR of 14

Archive directory: /export/home/vmr3/vmr3sys/data
Sample directory: auto_CustomQ_toc6_2004-02-19

Pulse Sequence: s2pul

Solvent: cdcl3
Temp: 25.0 C / 298.1 K
F1: 125.76 MHz
INNOVA-300 "SUNCDG51"

Relax. delay: 1.000 sec

Pulse: 45.0 degrees

Acq. time: 1.199 sec

Width: 19230.8 Hz

1024 repetitions

Observed C13, 75.4089480 MHz

DECOUPLE H1, 299.8897221 MHz

Power: 35 dB

continuously on

WALTZ-16 modulated

DATA PROCESSING

Line broadening: 1.0 Hz

F1 size: 65536

Total time: 37 min, 41 sec

¹H-NMR of 15

```

g585
ped2- run with findz0 before acquisition

Archive directory: /export/home/vmmr3/vmmr3sys/data
Sample directory: auto_custom0_1cc5_2004-02-25

Pulse Sequence: szpul

Solvent: cdcl3
Temp: 25.0 C / 298.1 K
File: P_Murphy_05-1
INVOA-300 "sunucdts1"

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.199 sec
Width 19230.8 Hz
0.254 s
4 repetitions
0.254 s
4 repetitions
DECODE 41.1 293.6897221 MHz
Power 35 dB
continuously on
MALTZ-16 modulated
LINE PROCESSING
Flt size 65536
Total time 37 min, 41 sec

```

5

¹H-NMR of 16

¹³C-NMR of 16

```
pcw-1 run with 100000 scans before acquisition
Archive directory: /export/home/vnmr3/vnmr3s/data
Sample directory: auto_Customq_loc6_2004-02-25
Pulse Sequence: szpu1
Solvent: cdcl3
Temp: 25.0 C / 298.1 K
File: P.-Murphy_06-1
INOVIA-300 "sunucddsi"
Relax: delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.199 sec
Width 19230.8 Hz
1024 repetitions
OBSERVE C13, 75.4069448 MHz
DECOUPLE H1, 299.8897221 MHz
Power 35.48
SOLVENT NOISE ON
WALTZ16
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 37 min, 41 sec
```

¹H-NMR of 17

¹³C-NMR of 17

^1H -NMR of 19

¹³C-NMR of 19

^1H -NMR of 20

¹³C-NMR of 20

$^1\text{H-NMR}$

^{13}C -NMR

^1H -NMR of 21

¹³C-NMR of 21

$^1\text{H-NMR}$

^{13}C -NMR

¹³C-NMR of 22

mt-fxn182-pure-carbon
Archive directory: /export/home/vnmr3/vnmr sys/data
Sample directory: auto_Custom0_10c30_2003-02-18
Pulse Sequence: szpul
Solvent: cdcl3 298.1 K
Temp: 300.2 K
File: P_Murphy_30.2
INOVA-300 "sunucds1"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.19 sec
Width 18850.1 Hz
2000 repetitions
OBSERVE C13, 75.4069355 MHz
DECOUPLE H1, 299.8897221 MHz
DECOUPLE H5, 299.8897221 MHz
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 1 hr, 13 min, 36 sec

^1H -NMR of 23

^{13}C -NMR of 23

Sample directory: auto_Custom0_loc1_2003-08-19
Pulse Sequence: szpul
Solvent: cdc13
Temp: 25.0 C / 298.1 K
File: P.-Murphy_01.2
INOVA-300 "sunucddsi1"
Relax: delay 1.000 sec
Pulse: 45.0 degrees
Acq: time 1.199 sec
Width 18850.1 Hz
256 repetitions
OBSERVE C13, 75.4069506 MHz
DECOUPLE H1, 299.6897221 MHz
Power 35 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
F2 75.4069506
Total time 9 min, 25 sec

Data from analysis of the stochastic dynamics simulations of 2-4

1. Glycosidic torsion analysis.

The glycosidic torsion angles of sampled structures were consistent with those found as low energy structures (<10 kJ/mol) above global minimum structure from conformational searches using SUMM method in Macromodel 8.5 for model disaccharides (OPLS-AA force field, GB/SA solvent continuum for water).

A. Glycosidic torsional profile for 2 for 1000 sampled structures

2. Glycosidic torsional profile for 3 for 1000 sampled structures

3. Glycosidic torsional profiles for 4 (1000 sampled structures)

4. Glycosidic torsion analysis from conformational searching techniques for model disaccharides

< 10 kJ/mol

5. Mannose Orientation Profiles as Figure 2 in Manuscript

Compound 2

Compound 3

Compound 4 **6. Analysis of Mannose-Mannose distance****A. Man-Man distance for Compound 2**

B. Mannose-Mannose distance for Compound 3 **C. Man-Man distance for 4**

7. Average Glucose/GlcA H1-C1-N-H Angles (Standard Deviation) from simulations

Compound	2	178.0°	(4.7)
Compound	3	175.5°	(3.2)
Compound	4	173.7°	(4.6)