Investigating the Elusive Mechanism of Glycosaminoglycan Biosynthesis

Xylophone V. Victor[1], Thao K. N. Nguyen[2], Manivannan Ethirajan[1], Vy M. Tran[2], Khiem V. Nguyen[1] and Balagurunathan Kuberan[1,2*]

[1]Department of Medicinal Chemistry and [2]Department of Bioengineering, University of Utah, Salt Lake City, UT 84112

Supplemental Section:

Structural data:

For xylosides 1 through 25, the structural data can be found in the following reference B. Kuberan et. al., (2008) *Chembiochem*, **9**, 198-200. The data for remaining xylosides are provided below.

Xyloside 26: 1H NMR (CD_3OD): δ 8.436 (1H, s, triazolyl H), 8.111 (1H, d, J = 7.815, Ar-H), 7.346 (1H, dd, J = 7.03, 8.55 Hz, Ar-H), 7.109 (1H, d, J = 8.2 Hz, Ar-H), 7.055 (1H, dd, J = 7.42, 8.59 Hz, Ar-H), 5.556 (1H, d, J = 9.38 Hz, H-1), 4.057-4.004 (2H, m, H-2, H-5a), 3.962 (3H, s), 3.745-3.682 (1H, m, H-4), 3.540-3.468 (2H, m, H-3, H-5b); Mass (EI): calculated for $C_{14}H_{17}N_3O_5$+H 308.12465, found 307.9333

Xyloside 27: 1H NMR (CD_3OD): δ 8.408 (1H, s, triazolyl H), 7.754 (2H, d, J = 8.99 Hz, Ar-H), 6.993 (2H, d, J = 8.98 Hz, Ar-H), 5.533 (1H, d, J = 9.37 Hz, H-1), 4.033 (1H, dd, J = 5.47, 11.33 Hz, H-5a), 3.937 (1H, d, J = 9.37 Hz, H-2), 3.824 (3H, s), 3.735-3.673 (1H, m, H-4), 3.574-3.464 (2H, m, H-3, H-5b); Mass (EI): calculated for $C_{14}H_{17}N_3O_5$ +H 308.12465, found 308.0667

Xyloside 28: 1H NMR (CD_3OD): δ 8.544 (1H, s, triazolyl H), 7.022 (2H, d, J = 2.34 Hz, Ar-H), 6.477 (1H, t, J = 2.34 Hz, Ar-H), 5.540 (1H, d, J = 9.37 Hz, H-1), 4.034 (1H, dd, J = 5.47, 11.33 Hz, H-5a), 3.937 (1H, d, J = 9.37 Hz, H-2), 3.824 (6H, s), 3.709-3.669 (1H, m, H-4), 3.541-3.465 (2H, m, H-3, H-5b); Mass (EI): calculated for $C_{15}H_{19}N_3O_6$ +H 338.13521, found 338.0000

Xyloside 29: 1H NMR (CD_3OD): δ 8.871 (1H, s, triazolyl H), 8.470 (2H, s, Ar-H), 7.949 (1H, s, Ar-H), 5.591 (1H, d, J = 9.38 Hz, H-1), 4.051 (1H, dd, J = 5.47, 11.32 Hz, H-5a), 3.937 (1H, d, J

= 9.38 Hz, H-2), 3.740-3.677 (1H, m, H-4), 3.554-3.482 (2H, m, H-3, H-5b); Mass (EI): calculated for $C_{15}H_{13}F_6N_3O_4$ +H 414.08885, found 413.9333

Xyloside 30: 1H NMR (CD_3OD): δ 8.50 (1H, s, triazolyl H), 8.25 (1H, dd, J = 3.51, 6.25 Hz, Ar-H), 7.93-7.95 (2H, m, Ar-H), 7.70 (1H, dd, *J* = 1.1, 7.0 Hz, Ar-H), 7.52-7.57 (3H, m, Ar-H), 5.64 (1H, d, *J* = 9.3 Hz, H-1), 4.07 (1H, dd, J = 5.47, 11.13 Hz, H-5a), 4.03 (1H, t, J = 9.37, H-2), 3.71-3.77 (1H, m, H-4), 3.51-3.58 (2H, m, H-3, H-5b); Mass (EI): calculated for $C_{17}H_{17}N_3O_4$ +H 328.12, found 327.93

Figure Legends

Figure S1. Calibration of size exclusion column with polystyrene sulfonate standards. Polystyrene sulfonate standards of various molecular weights, 65000 Da, were analyzed by size exclusion chromatography as described in the "Material and Methods" section. The migration times of the various polystyrene sulfonate species were plotted against the molecular weight to obtain a calibration curve. The migration time of GAG chains primed by various xylosides were compared to the calibration curve to determine the molecular weight.

Figure S2. Chain length analysis of xyloside-primed GAG chains. The molecular weight of the GAG chains synthesized on various primers was determined by measuring their migration time on size exclusion column as described in the "Material and Methods" section. V_0 and V_t represent the void volume and total volume, respectively. The average migration time was determined by using peak width at half maximum. The average molecular weight was determined using the migration time in comparison to the calibration curve obtained for polystyrene sulfonate standards performed under similar conditions.

Figure S3. Disaccharide profiles of xyloside-primed HS chains. GAG chains (~500,000 cpm) were digested with heparitinases and resulting disaccharides were analyzed by SAX-HPLC with inline flow scintillation analyzer as described in the Experimental section. The SAX elution chromatograms of representative of two independent experiments. I: ΔUA-GlcNAc; II: ΔUA-GlcNS; III: ΔUA-GlcNS6S; IV: ΔUA2S-GlcNS; V: ΔUA2S-GlcNAc6S and IV: ΔUA2S-GlcNS6S.

Figure S4. Disaccharide profiles of xyloside-primed DS chains. GAG chains (~500,000 cpm) were digested with chondroitinase ABC enzyme and resulting disaccharides were analyzed by SAX-HPLC with inline flow scintillation analyzer as described in the Experimental section. The SAX elution chromatograms of representative of two independent experiments. I: ΔUA-GalNAc; and II: ΔUA-GalNAc6S.

Figure S5. Disaccharide profiles of xyloside-primed CS chains. GAG chains (~500,000 cpm) were digested with chondroitinase ABC enzyme and resulting disaccharides were analyzed by SAX-HPLC with inline flow scintillation analyzer as described in the Experimental section. The SAX elution chromatograms of representative of two independent experiments. I: ΔUA-GalNAc; and II: ΔUA-GalNAc6S.

Figure S6. Long term priming ability of GAG chains by xyloside. The long term priming ability of click-xyloside was examined using xylosyl transferase deficient CHO cells (pgsA-745). 100,000 cells were seeded per well of 24-well plates and treated with xyloside **5** at 100 μM concentration in the presence of 50 μCi ^{35}S-SO_4^{2-} or D-[6-^{3}H]-glucosamine. The medium was removed from the well at 24, 48, 96 and 120 h, GAG chains were purified and quantified as described under "Experimental Methods".

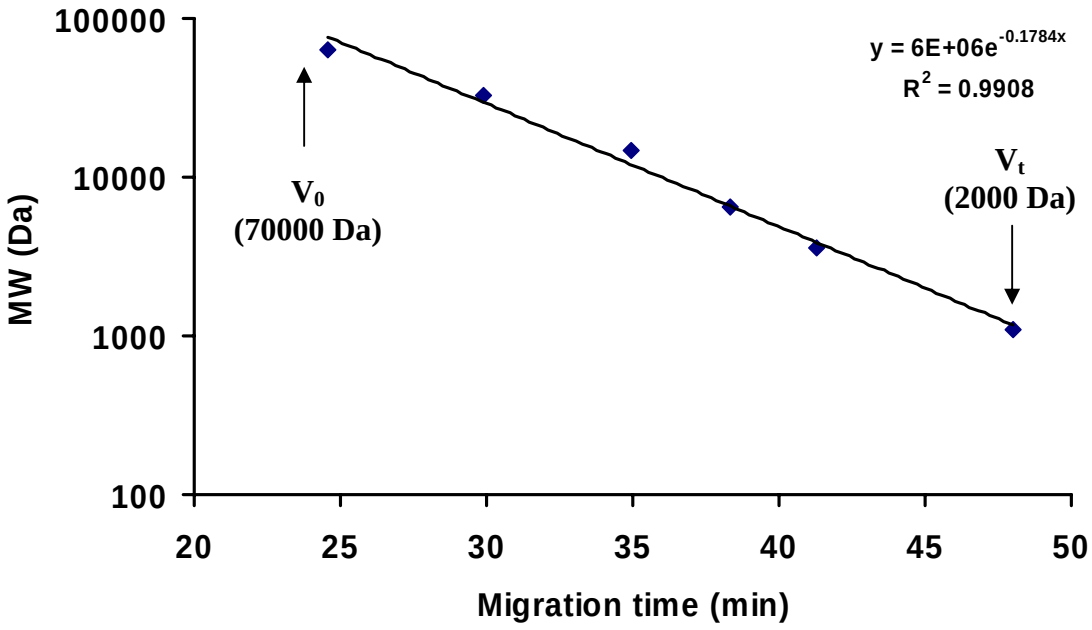
100000
10000
1000
100
MW (Da)
20
25
30
35
40
45
50
Migration time (min)
y = 6E+06e-0.1784x
R2 = 0.9908
V0
(70000 Da)
Vt
(2000 Da)

FIGURE S1

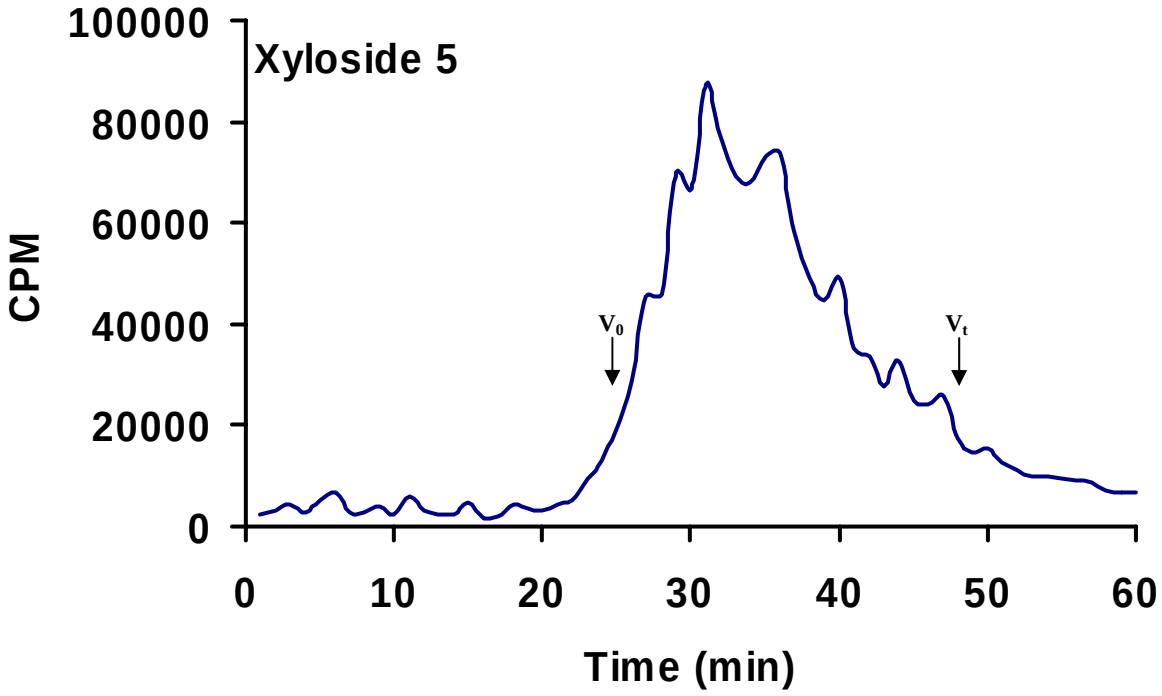
Xyloside 5
CPM
100000
80000
60000
40000
20000
0
0
10
20
30
40
50
60
Time (min)
V0
Vt

FIGURE S2A

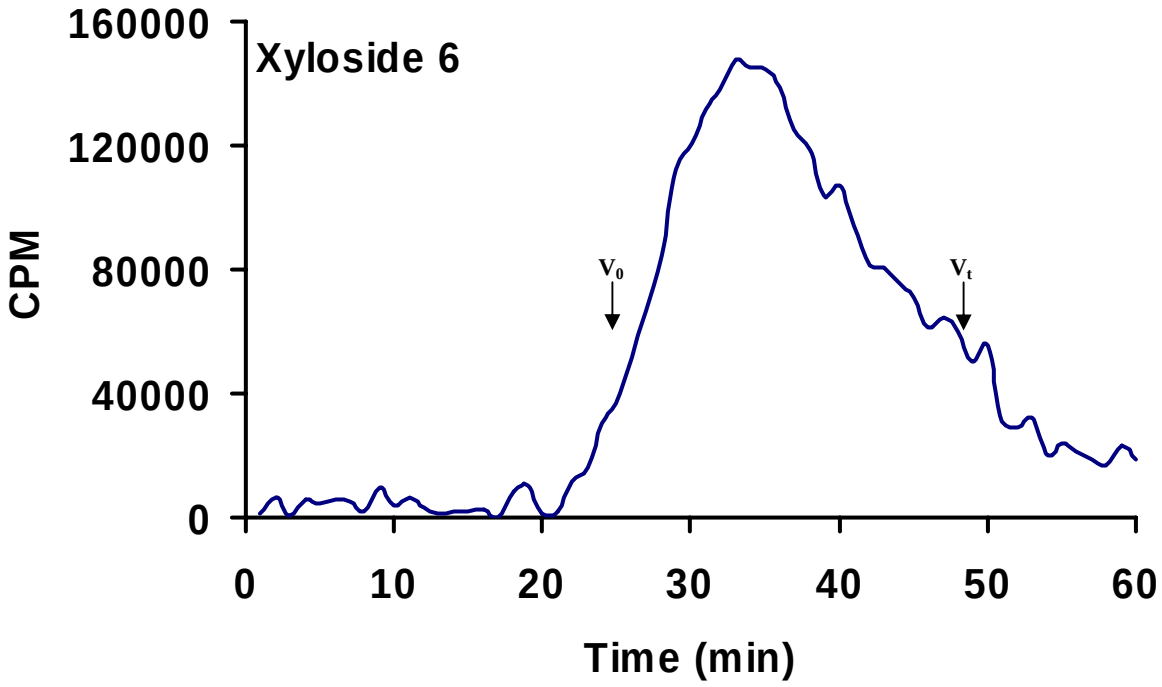
Xyloside 6
160000
120000
80000
40000
0
CPM
V_0
V_t
0
10
20
30
40
50
60
Time (min)

FIGURE S2B

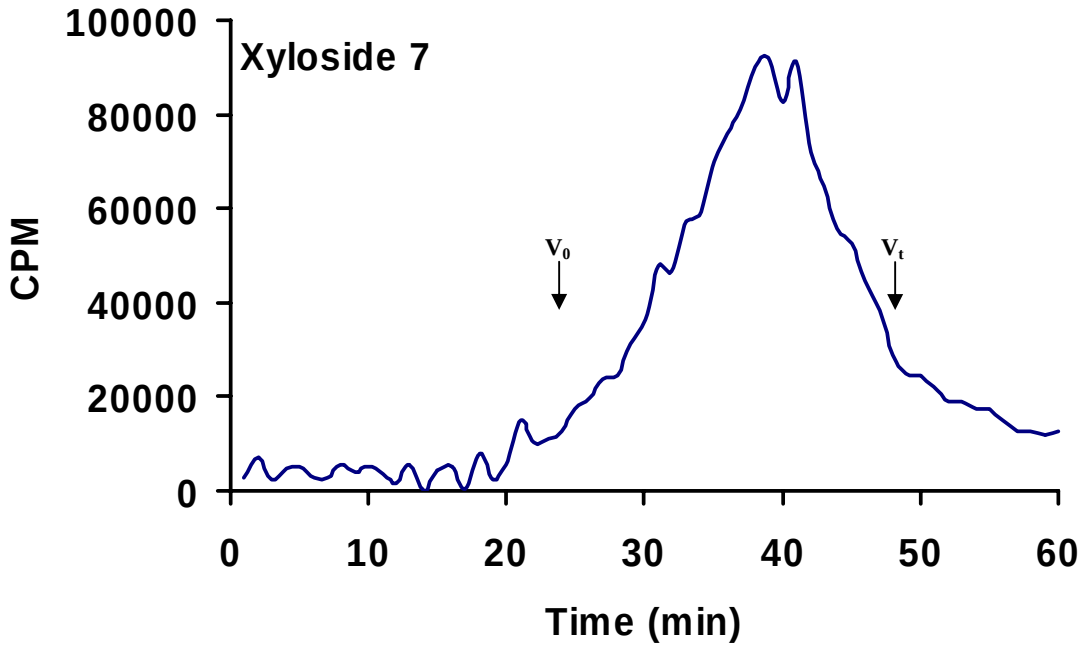
Xyloside 7
CPM
100000
80000
60000
40000
20000
0
0
10
20
30
40
50
60
Time (min)
V0
Vt

FIGURE S2C

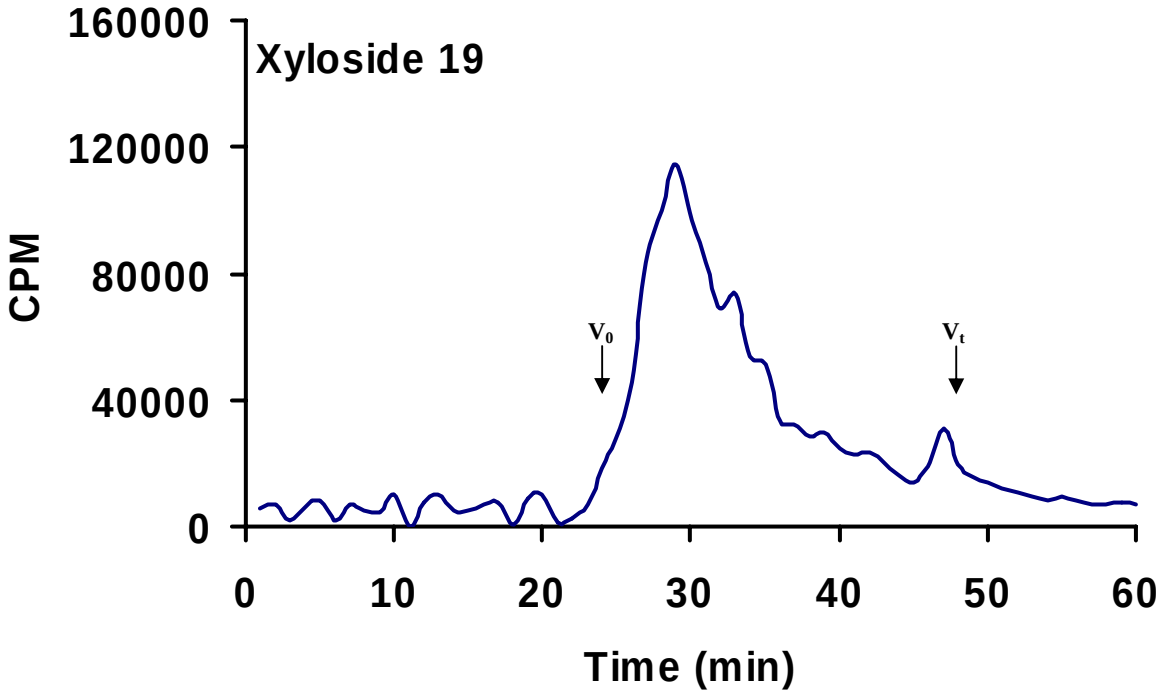
Xyloside 19
160000
120000
80000
40000
0
CPM
V_0
V_t
0
10
20
30
40
50
60
Time (min)

FIGURE S2D

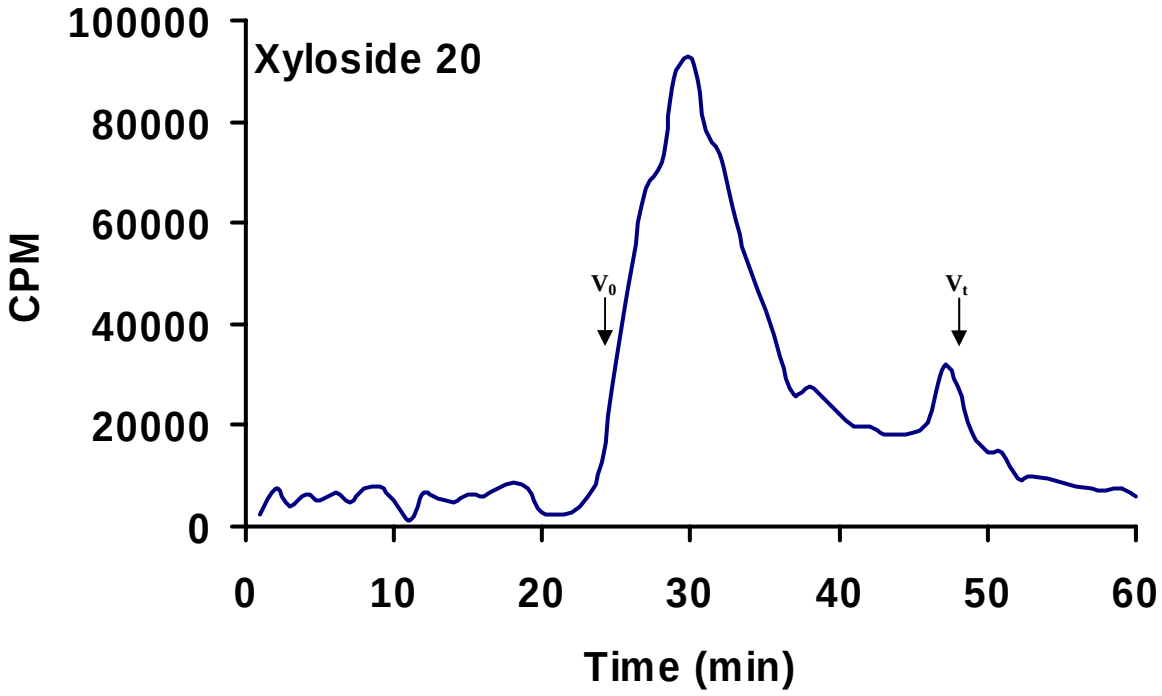
Xyloside 20
CPM
100000
80000
60000
40000
20000
0
V_0
V_t
0
10
20
30
40
50
60
Time (min)

FIGURE S2E

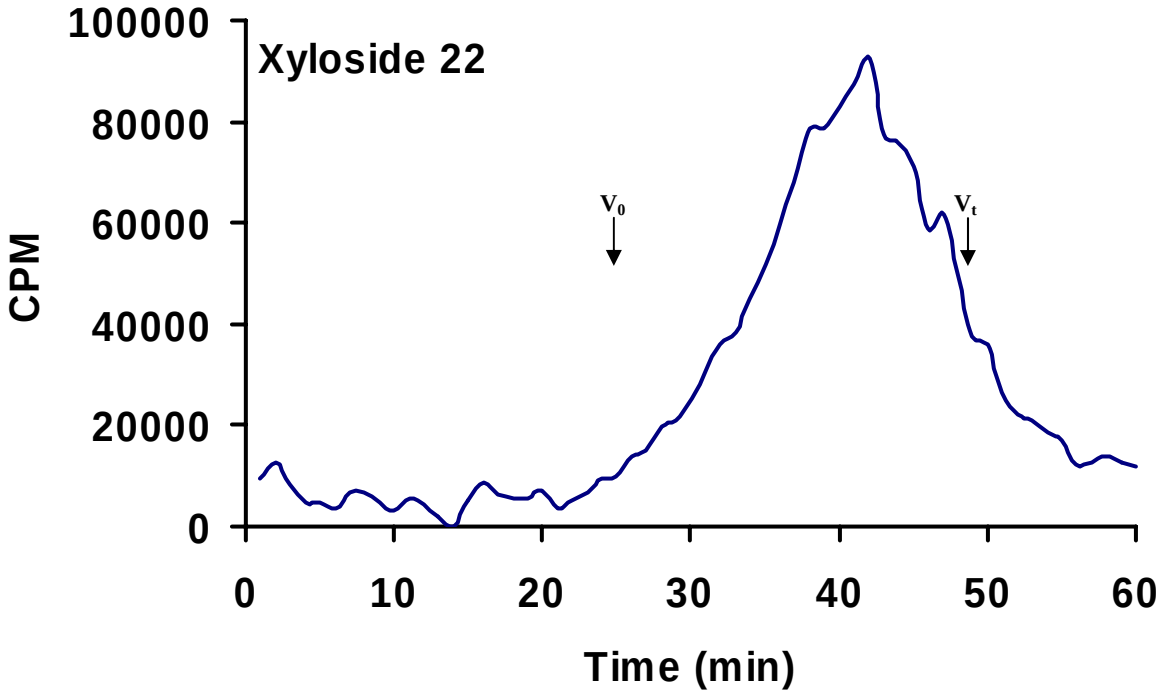
Xyloside 22
CPM
100000
80000
60000
40000
20000
0
V_0
V_t
0
10
20
30
40
50
60
Time (min)

FIGURE S2F

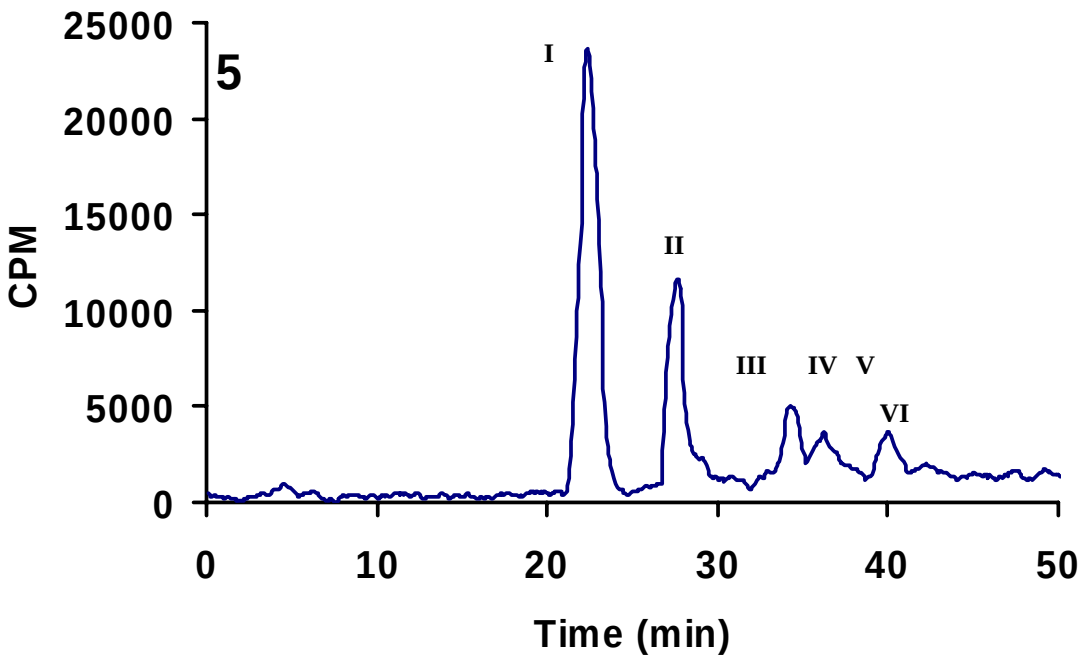
25000
20000
15000
10000
5000
0
CPM
5
I
II
III
IV
V
VI
0
10
20
30
40
50
Time (min)

FIGURE S3A

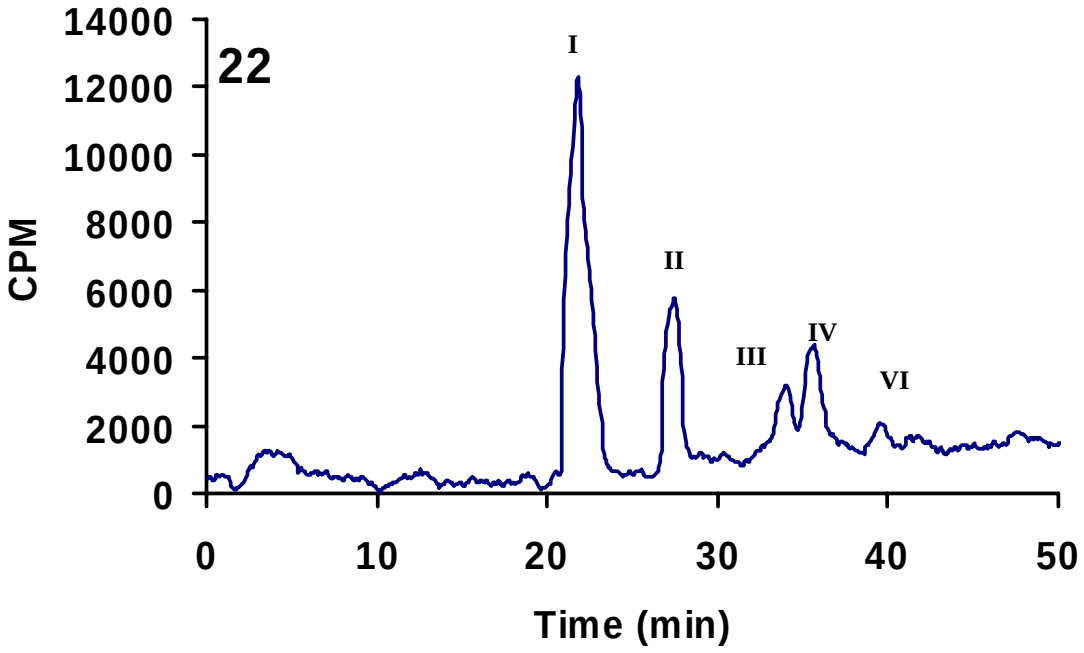
22
I
II
III
IV
VI
CPM
14000
12000
10000
8000
6000
4000
2000
0
0
10
20
30
40
50
Time (min)

FIGURE S3B

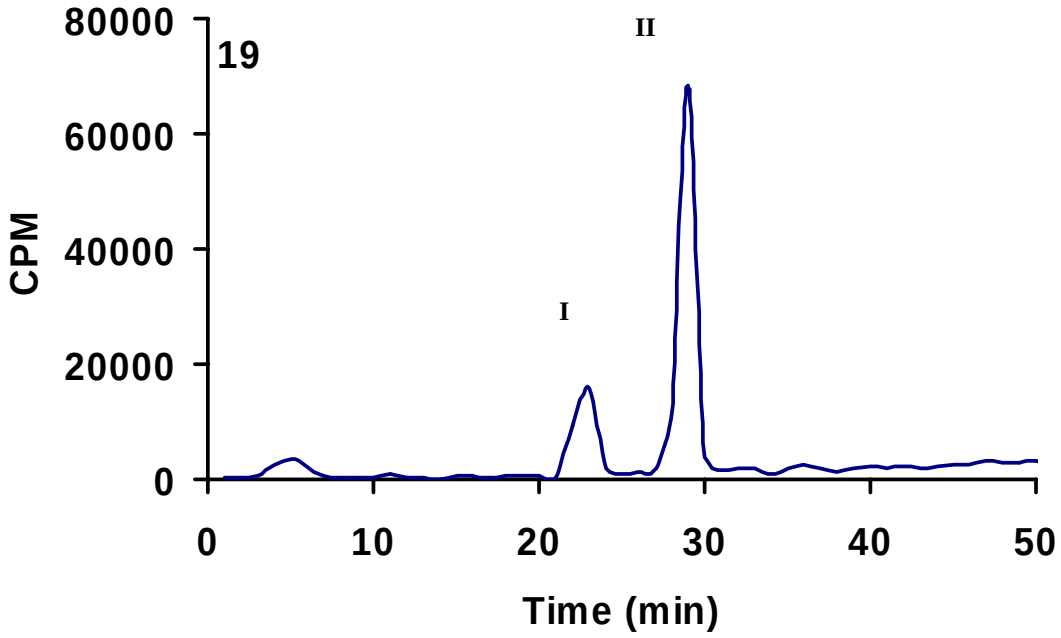

FIGURE S4A

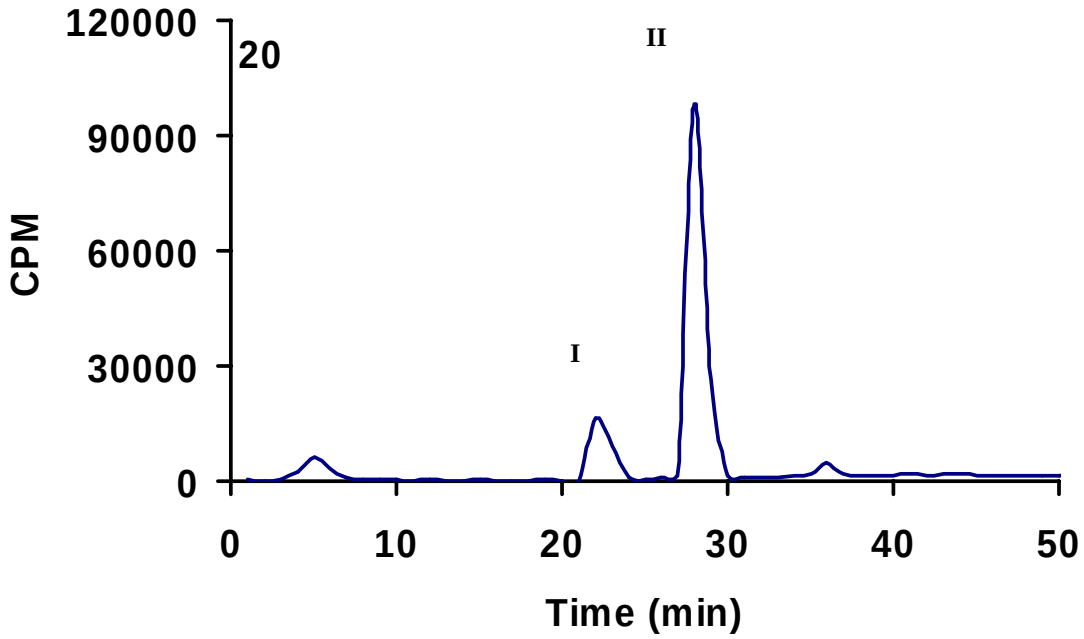

FIGURE S4B

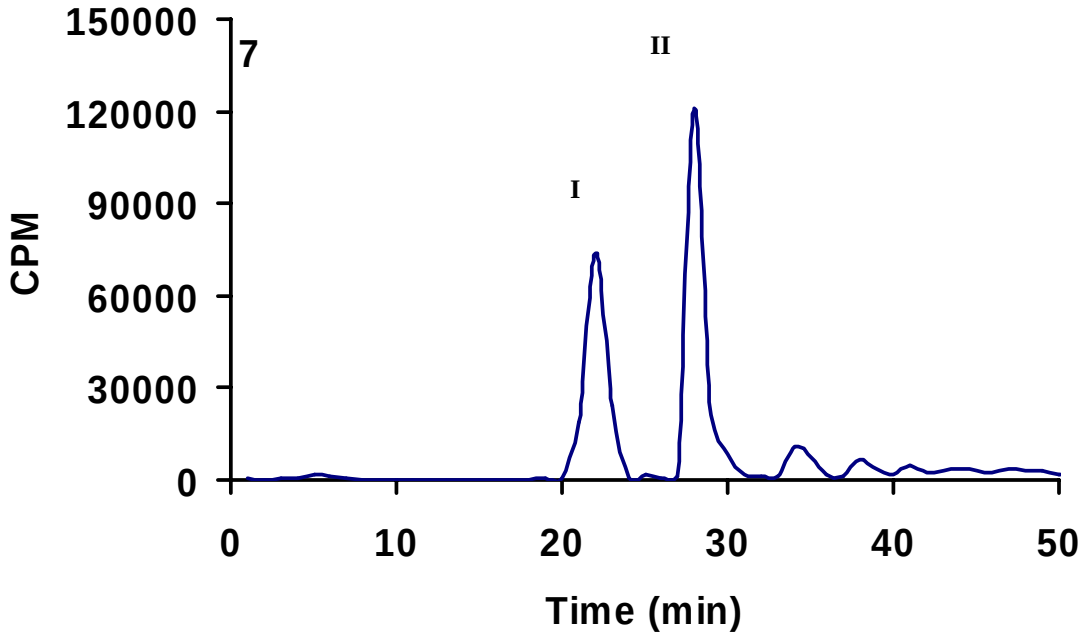
150000
120000
90000
60000
30000
0
CPM
7
I
II
0
10
20
30
40
50
Time (min)

FIGURE S5A

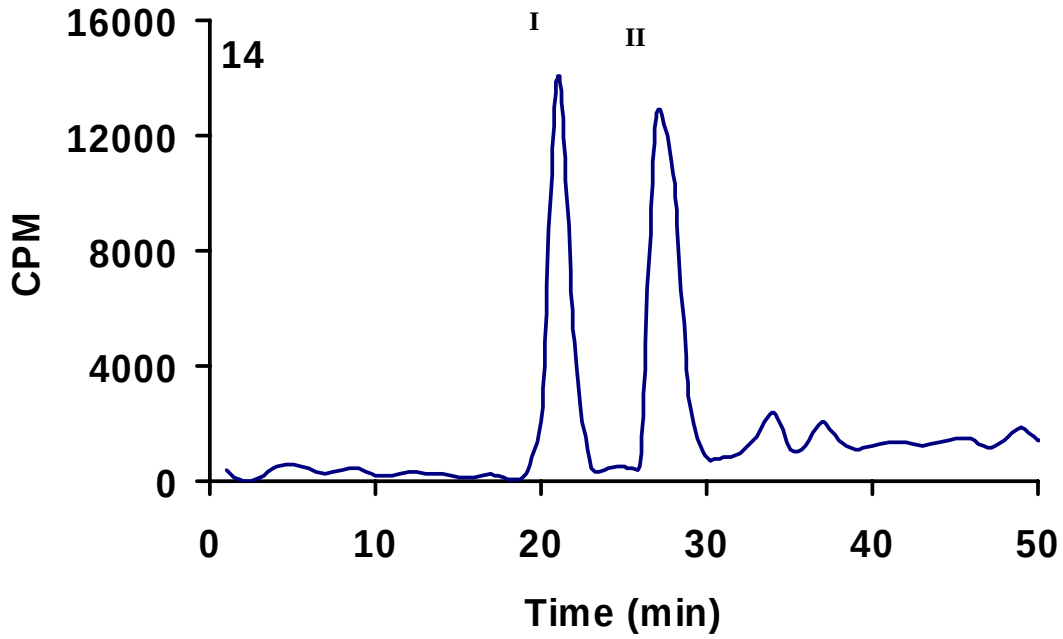

FIGURE S5B

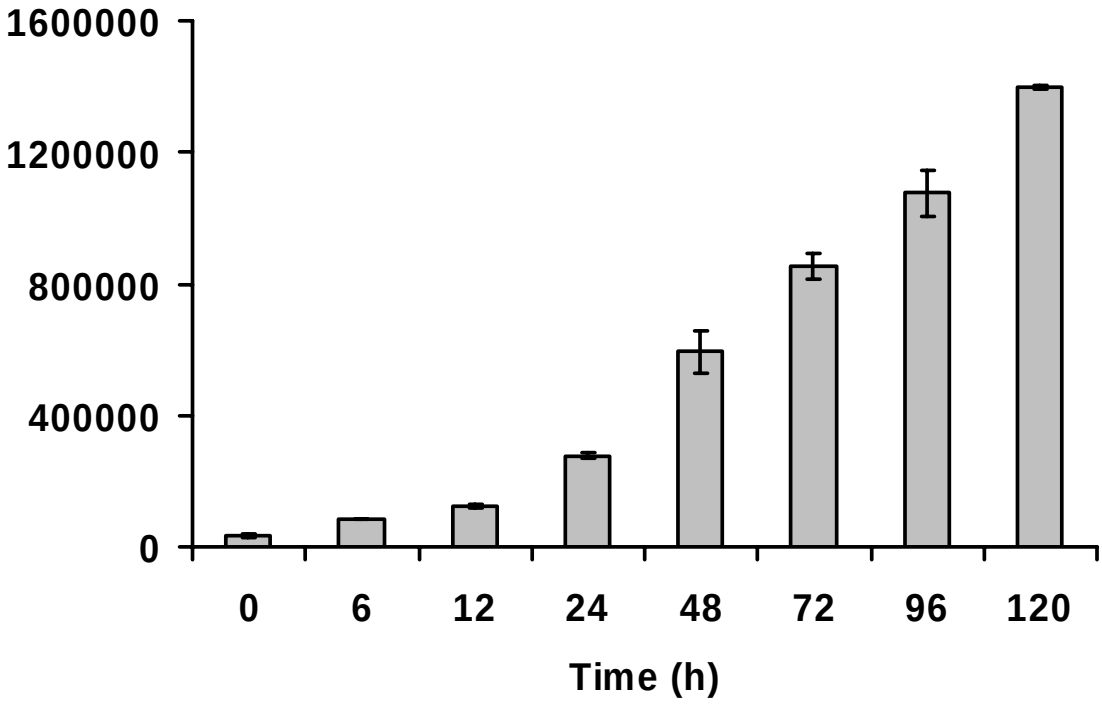

FIGURE S6