Measuring deuterium enrichment of glucose hydrogen atoms by gas chromatography mass spectrometry

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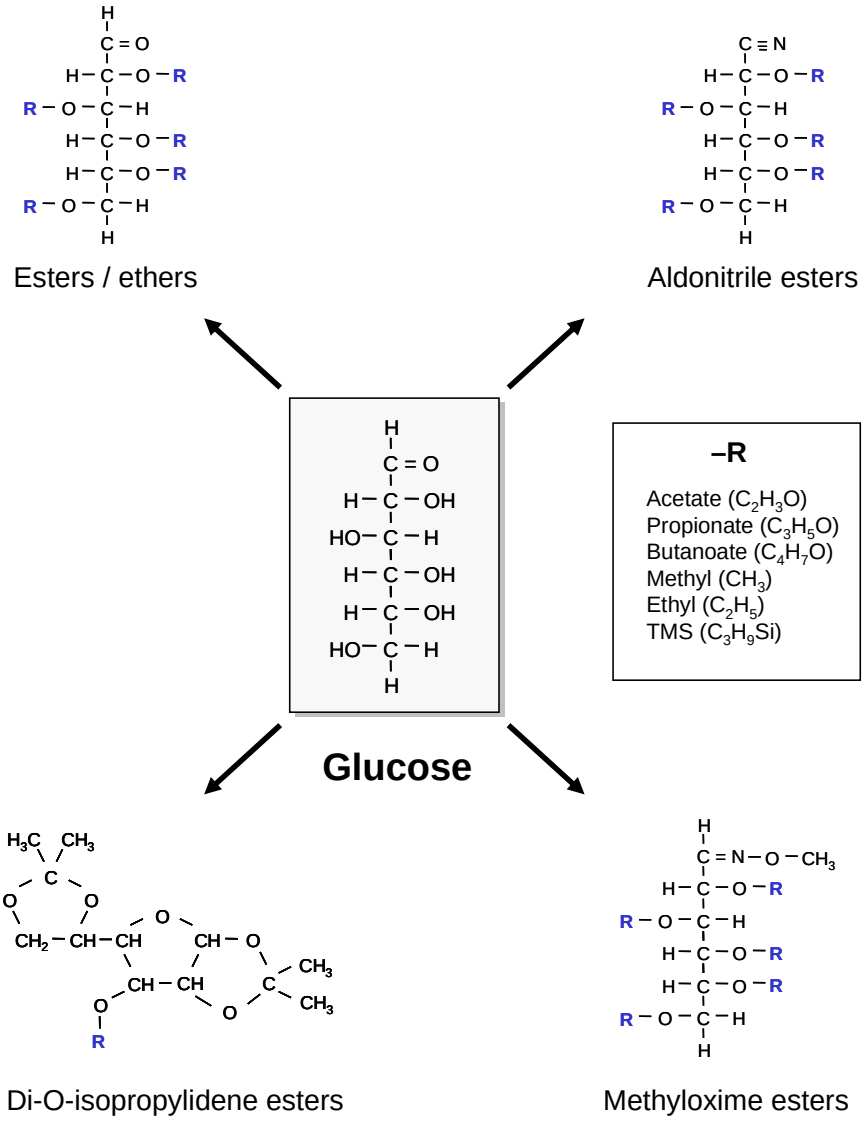

Figure S-1. Schematic overview of the two step procedure for preparation of glucose derivatives. In the first reaction step the carbonyl group at C1 of glucose may be derivatized, and in the second reaction step hydroxyl groups of glucose are derivatized. Based on this procedure 18 different glucose derivatives were prepared and analyzed by GC/MS.

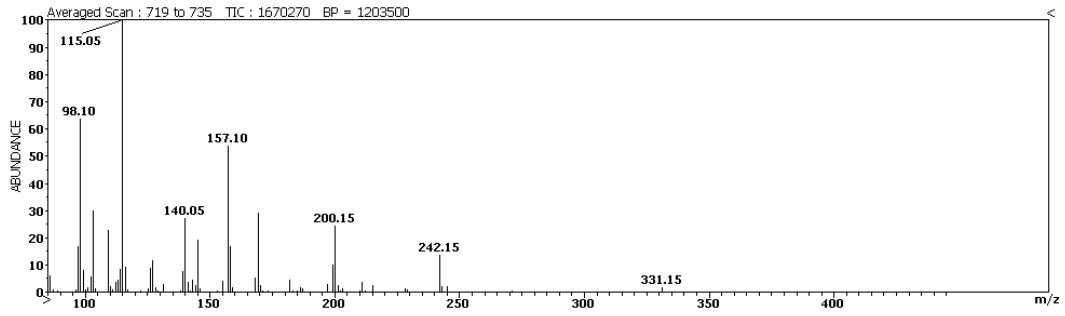

Figure S-2a. Electron impact mass spectrum of pentaacetate glucose

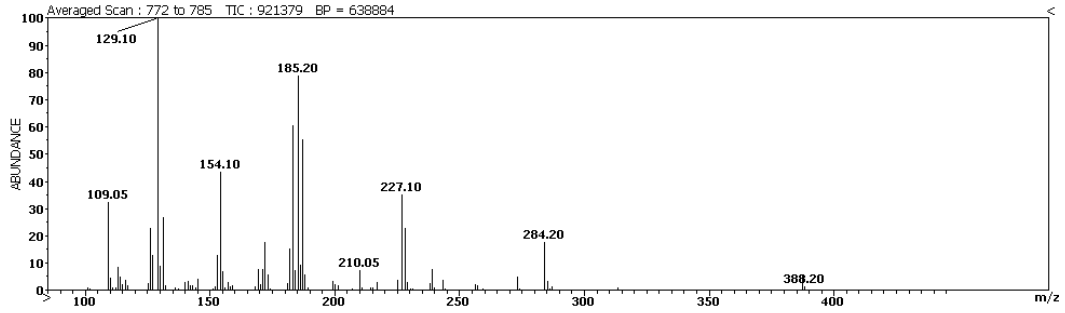

Figure S-2b. Electron impact mass spectrum of pentapropionate glucose

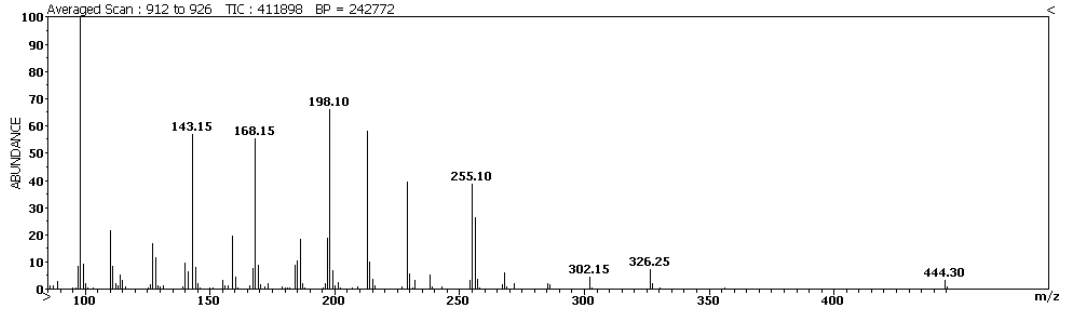

Figure S-2c. Electron impact mass spectrum of pentabutanoate glucose

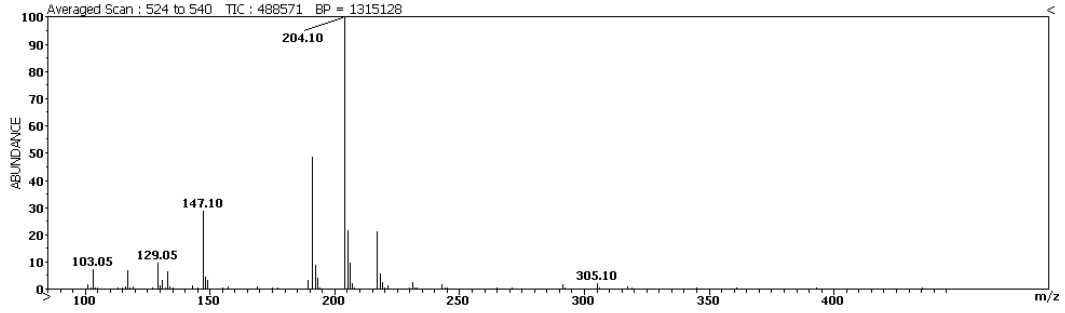

Figure S-2d. Electron impact mass spectrum of pentatrimethylsilyl glucose

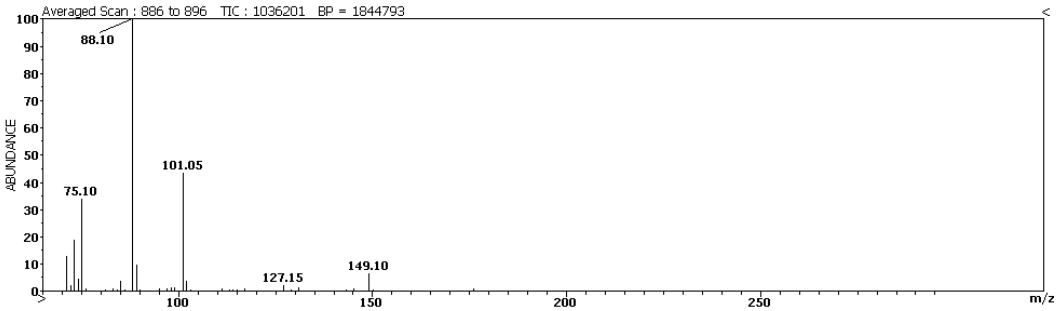

Figure S-2e. Electron impact mass spectrum of permethyl glucose

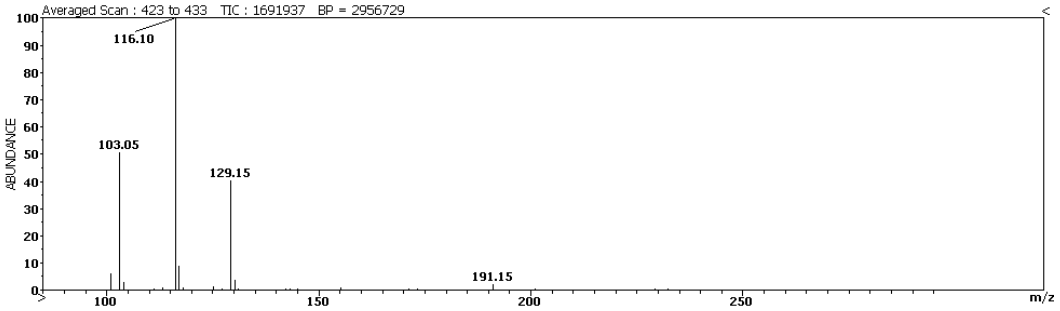

Figure S-2f. Electron impact mass spectrum of perethyl glucose

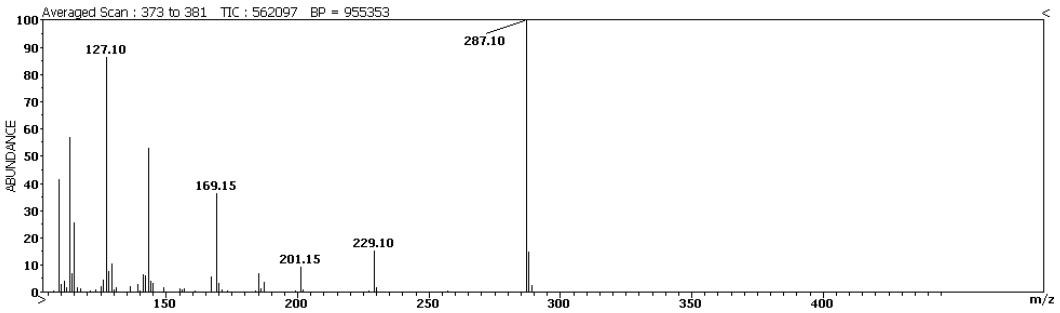

Figure S-2g. Electron impact mass spectrum of di-O-isopropylidene acetate glucose

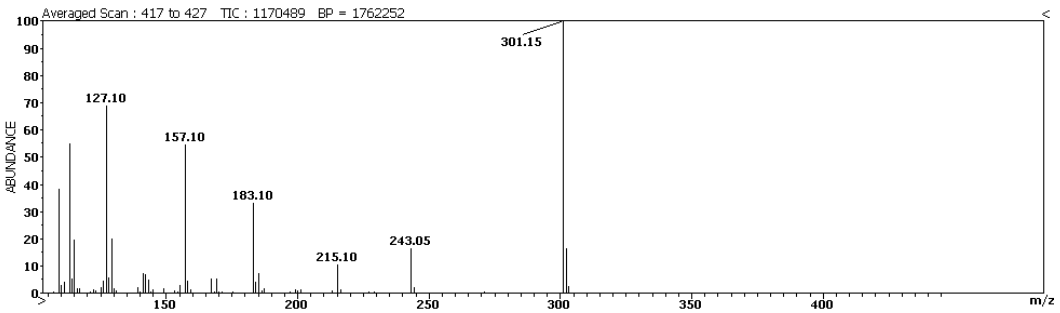

Figure S-2h. Electron impact mass spectrum of di-O-isopropylidene propionate glucose

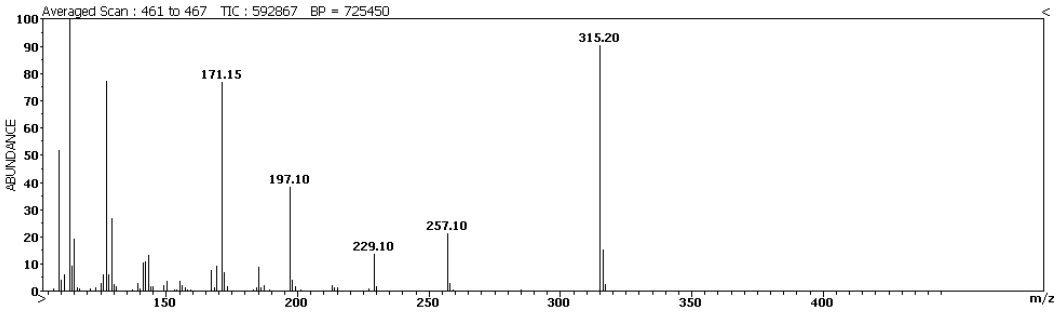

Figure S-2i. Electron impact mass spectrum of di-O-isopropylidene butanoate glucose

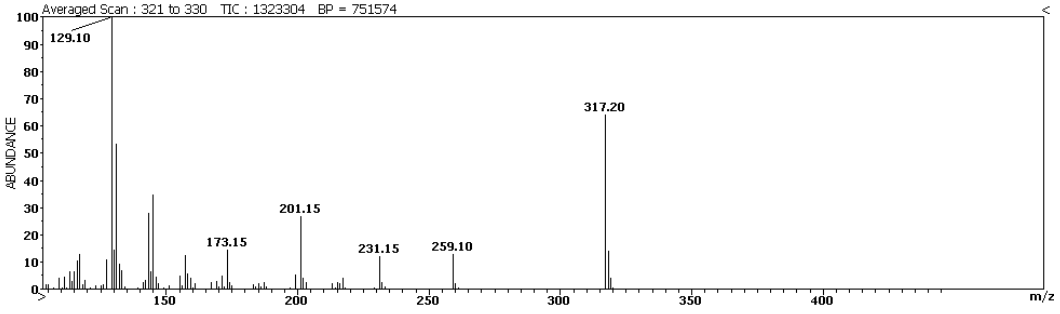

Figure S-2j. Electron impact mass spectrum of di-O-isopropylidene trimethylsilyl glucose

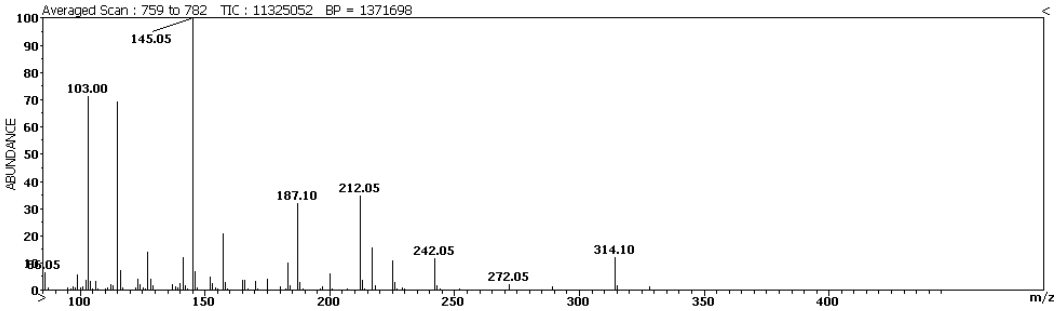

Figure S-2k. Electron impact mass spectrum of aldonitrile pentaacetate glucose

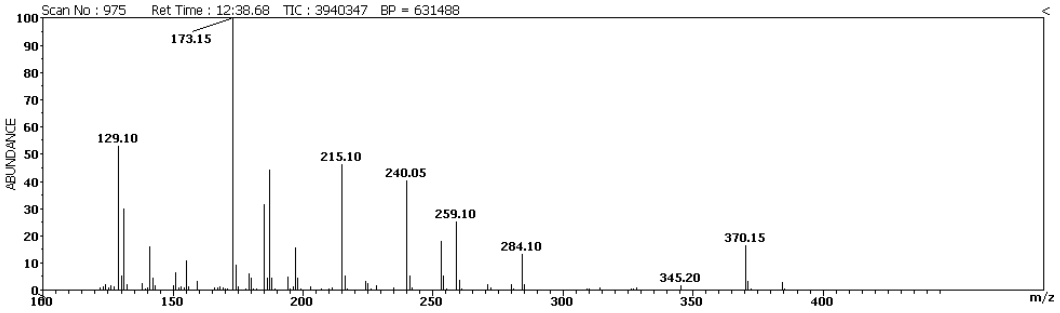

Figure S-2l. Electron impact mass spectrum of aldonitrile pentapropionate glucose

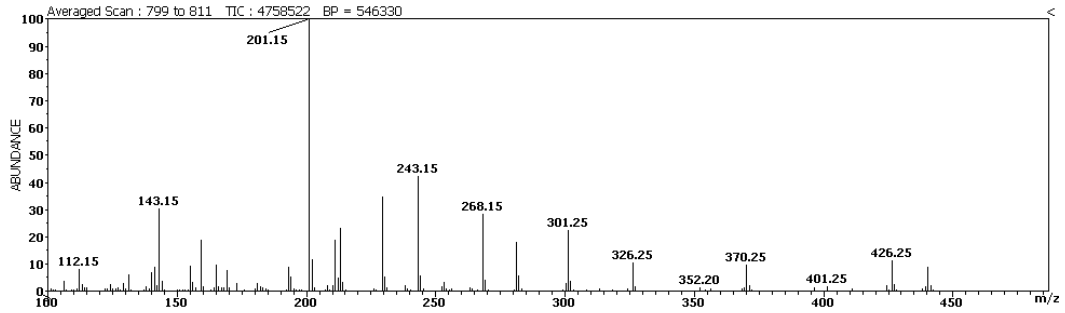

Figure S-2m. Electron impact mass spectrum of aldonitrile pentabutanoate glucose

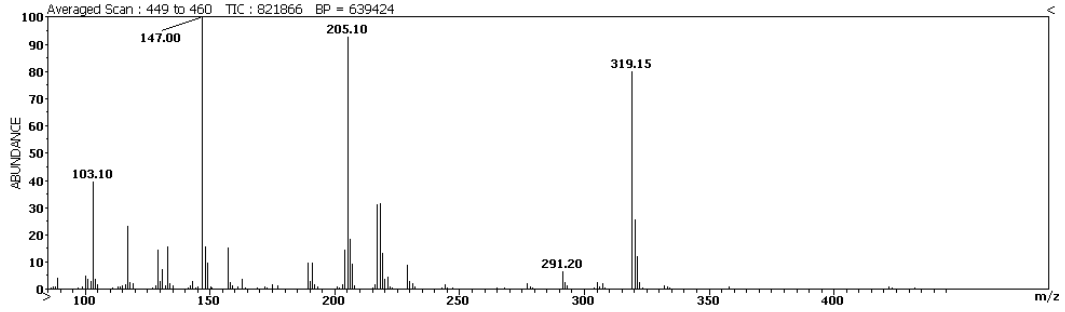

Figure S-2n. Electron impact mass spectrum of aldonitrile pentatrimethylsilyl glucose

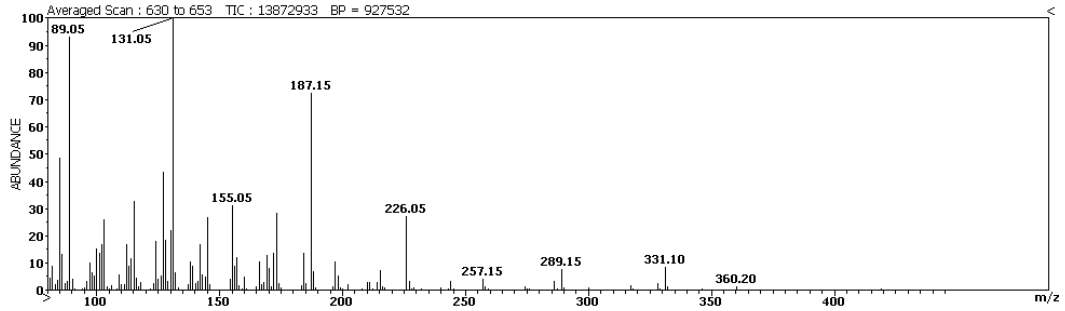

Figure S-2o. Electron impact mass spectrum of methyloxime pentaacetate glucose

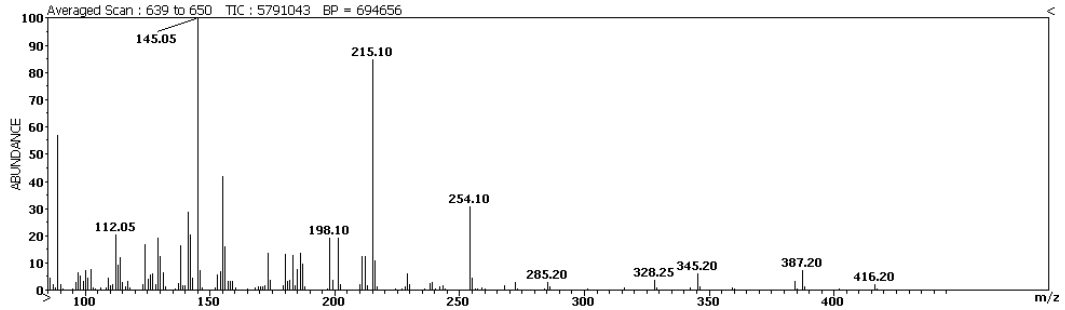

Figure S-2p. Electron impact mass spectrum of methyloxime pentapropionate glucose

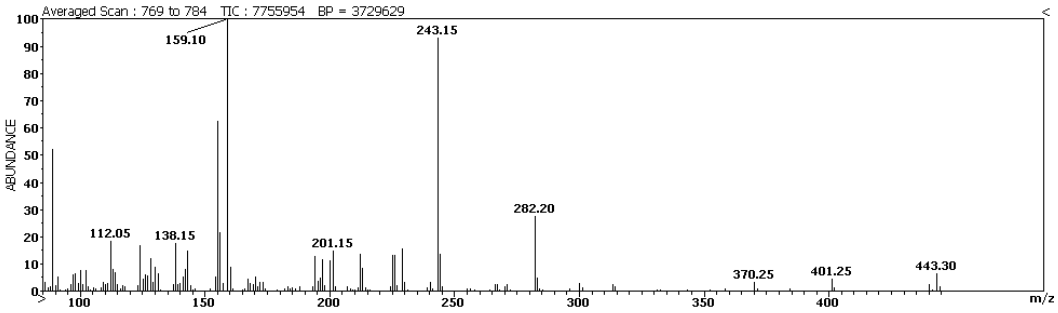

Figure S-2q. Electron impact mass spectrum of methyloxime pentabutanoate glucose

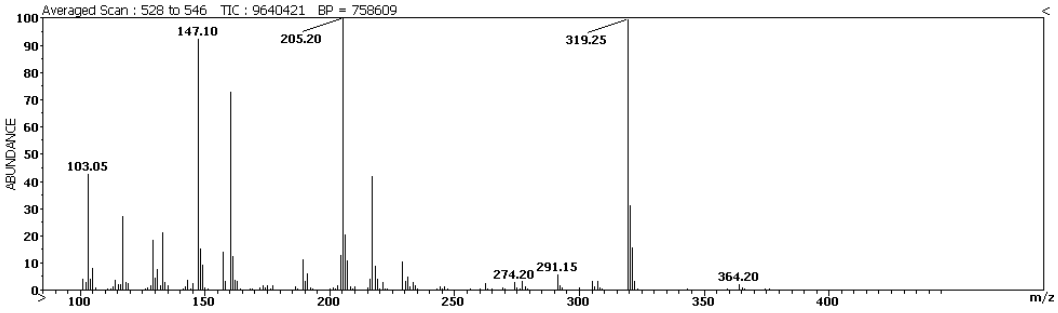

Figure S-2r. Electron impact mass spectrum of methyloxime pentatrimethylsilyl glucose

Table S-3. List of most abundant fragments for each glucose derivative that were evaluated.

Glucose derivative	Most abundant fragments (*m/z*)
Pentaacetate	98, 103, 109, 115, 140, 145, 157, 169, 200, 242, 331
Pentapropionate	109, 129, 131, 154, 183, 185, 187, 210, 227, 284, 387
Pentabutanoate	98, 110, 127, 143, 159, 168, 186, 198, 213, 229, 255, 302, 326, 444
Pentatrimethylsilyl	103, 117, 129, 133, 147, 191, 204, 217, 305
Permethyl	75, 88, 101, 149
Perethyl	103, 116, 129, 191
Di-O-isopropylidene acetate	109, 113, 127, 143, 169, 185, 201, 229, 287
Di-O-isopropylidene propionate	109, 113, 127, 157, 183, 215, 243, 301
Di-O-isopropylidene butanoate	109, 113, 127, 171, 197, 229, 257, 315
Di-O-isopropylidene trimethylsilyl	129, 131, 143, 145, 173, 185, 201, 231, 259, 317
Aldonitrile pentaacetate	103, 115, 127, 141, 145, 157, 187, 212, 217, 225, 242, 272, 314
Aldonitrile pentapropionate	129, 131, 141, 155, 173, 185, 187, 197, 215, 240, 253, 259, 284, 345, 370, 384
Aldonitrile pentabutanoate	112, 143, 155, 159, 169, 193, 201, 211, 213, 229, 243, 268, 281, 301, 326, 370, 426, 440
Aldonitrile pentatrimethylsilyl	103, 117, 129, 133, 147, 157, 189, 191, 205, 217, 229, 291, 319
Methyloxime pentaacetate	85, 89, 115, 127, 131, 145, 155, 173, 187, 197, 215, 226, 257, 286, 289, 331
Methyloxime pentapropionate	89, 112, 141, 145, 155, 198, 201, 215, 254, 328, 345, 384, 387, 416
Methyloxime pentabutanoate	89, 112, 124, 138, 155, 159, 212, 229, 243, 282, 370, 401, 443
Methyloxime pentatrimethylsilyl	103, 117, 129, 133, 147, 160, 189, 205, 217, 229, 291, 319, 364

Table S-4. Mass isotopomer distributions for deuterated glucose standards.

m/z	M+0	M+1	M+2	M+3	M+4
[1-^{2}H]glucose					
301	0.0	82.1	15.1	2.5	0.3
145	0.2	89.4	9.4	1.0	0.1
173	90.6	8.2	1.1	0.1	0.0
259	86.5	11.6	1.8	0.2	0.0
284	82.7	14.9	2.2	0.2	0.1
370	78.8	17.5	3.3	0.4	0.0
[2-^{2}H]glucose					
301	0.0	83.1	14.3	2.4	0.2
145	0.8	90.4	7.8	0.9	0.1
173	90.5	8.3	1.1	0.1	0.0
259	86.3	11.7	1.9	0.2	0.0
284	0.8	84.6	12.6	1.9	0.2
370	0.8	80.7	15.4	2.8	0.4
[3-^{2}H]glucose					
301	1.3	81.3	14.7	2.4	0.3
145	90.0	8.9	1.0	0.1	0.0
173	90.5	8.3	1.1	0.1	0.0
259	86.3	11.6	1.9	0.2	0.0
284	1.1	83.3	13.3	2.0	0.2
370	0.9	79.8	16.1	3.0	0.4
[4-^{2}H]glucose					
301	4.1	80.8	12.7	2.1	0.2
145	87.5	11.2	1.2	0.1	0.0
173	90.4	8.3	1.1	0.1	0.0
259	6.1	81.1	10.9	1.7	0.2
284	5.1	80.9	12.0	1.8	0.2
370	4.5	77.5	15.0	2.7	0.3

Table S-4 (continued). Mass isotopomer distributions of selected fragments for deuterated glucose standards.

m/z	M+0	M+1	M+2	M+3	M+4
[5-^{2}H]glucose					
301	0.0	84.1	13.4	2.2	0.2
145	91.3	7.7	1.0	0.1	0.0
173	0.0	90.6	8.3	1.1	0.1
259	0.0	86.4	11.6	1.8	0.2
284	85.0	12.9	1.9	0.2	0.0
370	0.0	81.0	15.7	2.9	0.4
[6,6-$^{2}H_2$]glucose					
301	0.1	2.9	79.3	14.9	2.6
145	91.3	7.0	1.6	0.2	0.0
173	0.6	1.5	86.2	10.4	1.3
259	0.3	2.3	81.8	13.5	2.2
284	84.8	12.8	2.2	0.2	0.0
370	78.6	17.5	3.3	0.4	0.2

Table S-5. Mass isotopomer distributions for mixtures of [2-^{2}H]-, [5-^{2}H]glucose and natural glucose.

m/z	M+0	M+1	M+2	M+3	M+4
[2-^{2}H]glucose + [5-^{2}H]glucose (50:50)					
301	0.0	83.1	14.3	2.4	0.3
145	46.6	48.7	4.2	0.5	0.0
173	44.5	50.1	4.8	0.6	0.0
259	42.3	49.8	6.9	1.0	0.1
284	44.1	47.8	7.1	1.0	0.1
370	0.1	81.0	15.7	2.9	0.3
[2-^{2}H]glucose + [5-^{2}H]glucose + natural glucose (10:10:80)					
301	68.4	26.7	4.3	0.6	0.0
145	83.3	15.1	1.4	0.1	0.0
173	81.3	16.7	1.9	0.2	0.0
259	77.4	19.4	2.8	0.3	0.0
284	77.0	19.5	2.9	0.4	0.1
370	65.4	28.4	5.3	0.8	0.1
[2-^{2}H]glucose + [5-^{2}H]glucose + natural glucose (5:5:90)					
301	76.0	20.2	3.3	0.4	0.0
145	87.6	11.2	1.1	0.1	0.0
173	85.7	12.6	1.5	0.1	0.0
259	81.9	15.6	2.3	0.3	0.0
284	80.8	16.1	2.5	0.4	0.2
370	72.9	22.3	4.1	0.6	0.1
[2-^{2}H]glucose + [5-^{2}H]glucose + natural glucose (1:1:98)					
301	82.4	14.9	2.5	0.3	0.0
145	91.5	7.7	0.7	0.1	0.0
173	89.6	9.1	1.2	0.1	0.0
259	85.2	12.7	2.0	0.2	0.0
284	84.2	13.3	2.2	0.2	0.1
370	79.2	17.2	3.2	0.4	0.0

S-6. Calculation of positional deuterium enrichments using least-squares regression.

The amount of deuterium at each carbon position of glucose was determined by least-squares regression. First, theoretical MIDs were calculated for the six selected glucose fragments and all isotopomers of glucose. With seven stable (i.e. carbon bound) hydrogen atoms, there are 128 (=2^7) possible isotopomers of glucose hydrogen atoms. The MID of a fragment of glucose can be viewed as a linear combination of theoretical MIDs of each isotopomer of glucose, with isotopomer fractions (x) as the coefficients:

$$MID = x_{0000000} \times MID^{theor.}_{0000000} + x_{0000001} \times MID^{theor.}_{0000001} + \cdots + x_{1111111} \times MID^{theor.}_{1111111} \quad (1)$$

The above equation can be written it matrix form:

$$MID = [MID^{theor.}_{0000000} \quad MID^{theor.}_{0000001} \quad \ldots \quad MID^{theor.}_{1111111}] \times x \quad (2)$$

With six glucose fragments, we have six of such equations, which can be written in a matrix form:

$$\begin{bmatrix} MID^{frag.\ 1} \\ MID^{frag.\ 2} \\ \ldots \\ MID^{frag.\ 6} \end{bmatrix} = \begin{bmatrix} MID^{theor.\ frag.\ 1}_{0000000} & MID^{theor.\ frag.\ 1}_{0000001} & \ldots & MID^{theor.\ frag.\ 1}_{1111111} \\ MID^{theor.\ frag.\ 2}_{0000000} & MID^{theor.\ frag.\ 3}_{0000001} & \ldots & MID^{theor.\ frag.\ 2}_{1111111} \\ \ldots & \ldots & \ldots & \ldots \\ MID^{theor.\ frag.\ 6}_{0000000} & MID^{theor.\ frag.\ 6}_{0000001} & \ldots & MID^{theor.\ frag.\ 6}_{1111111} \end{bmatrix} \times x \quad (3)$$

Or, in short notation:

$$MIDs = MIDs^{theor.} \times x \quad (4)$$

Isotopomer fractions (x) were estimated by solving the following regression problem using Matlab (Mathworks Inc.):

$$\min \sum (\text{MIDs} - \text{MIDs}^{\text{measured}})^2 \quad (5)$$

$$\text{s.t. } \text{MIDs} = \text{MIDs}^{\text{theoretical}} \times x$$

$$0 \le x_i \le 1, \Sigma x_i = 1$$

The deuterium enrichments of glucose hydrogen atoms were then determined by summation of appropriate isotopomer fractions, where N is a 6×128 matrix:

$$[D_1, D_2, D_3, D_4, D_5, D_{66}] = N \times x \quad (6)$$

We denote the deuterium enrichment at C1 of glucose as D_1, the enrichment at C2 as D_2, etc. Since there are two hydrogen atoms at C6 that cannot be distinguished by GC-MS we determined the average enrichment at C_6 as $D_{66}/2$.

Calculation of theoretical mass isotopomer distributions

The theoretical MIDs for each glucose fragment and all isotopomers of glucose in Eq. (3) were calculated using the elementary metabolite unit (EMU) methodology (Antoniewicz et al, Metab Eng 9(1): 68-86, 2007). For calculating theoretical MID the following natural isotope abundances were used: ^{2}H (0.0156 At%), ^{13}C (1.082 At%), ^{15}N (0.366 At%), ^{17}O (0.038 At%), ^{18}O (0.204 At%), ^{29}Si (4.69 At%), ^{30}Si (3.09 At%).