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Neoglycopeptides through direct functionalization of cysteine

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ABSTRACT

Neoglycopeptides are readily prepared by direct functionalization of cysteine-containing peptides followed by click triazole formation between the resulting propargylated peptides and protected or free (2-azido)-ethyl gluco-, manno,- and galactopyranosides.

1. Introduction

Post-translational protein glycosylation is an important biological process which plays a tremendous role in the conformation and/or biological activity of the protein. Mimicking this type of enzymatic reaction in a flask using chemical manipulation is still a challenge.[1] The search for general, efficient, and without side-product chemical reactions to graft two biomolecules has been intense in the last decade. This culminated with the discovery of the so-called chemical ligation processes, such as native chemical ligation,[2] Staudinger ligation,[3] 'click' Huisgen reaction etc.[4] Most of these methods require modifications of the biomolecules to link.

In connection with a program of labeling glycopolypeptides and glycoproteins using ^{18}F labeled sugars, we needed a fast sugar–peptide ligation process. We reasoned that biologically significant peptides or proteins often contain a cysteine residue not embedded in a disulfide bond. We planned to take advantage of the presence of the cysteine thiol reactivity.[5] The high nucleophilicity of the thiol function of this unique amino-acid can be exploited to prepare S-alkylated derivatives.[6] Ideally, this operation should be carried out on a free peptide and would provide a single functionalized peptide with an anchoring point allowing the connection of a properly derivatized sugar. For example, the introduction of a propargyl group on cysteine would allow its subsequent use in click triazole formation. To the best of our knowledge, this possibility of exploiting *S*-propargyl-cysteine derivatives for connection to a sugar has not been used until recently. An elegant solution has recently been proposed by Dondoni[7] to connect 1-thiosugars with cysteine-containing peptides. This prompts us to disclose part of our own results along this line.

The synthetic blueprint for connecting a peptide and a sugar is depicted in Figure 1. The first step is the selective S-propargylation of the peptide. The second step involves a copper catalyzed Huisgen reaction, one of the so-called 'click chemistry' reactions, the efficiency of which, is now well established as a fast and traceless ligation process.[8–10]

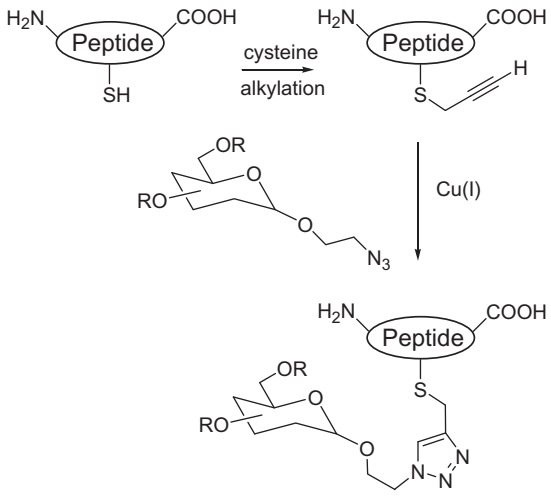

Figure 1. Synthetic strategy for neoglycopeptides construction.

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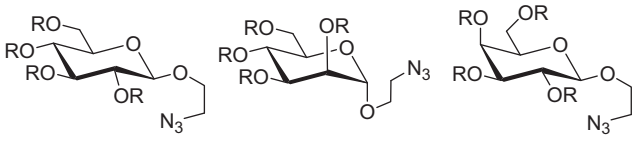
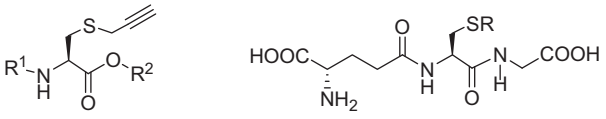


1 R = OAc (65%) **3** R = OAc (65%) **5** R = OAc (60%)
2 R = H **4** R = H **6** R = H

Figure 2. Structures of azido sugar derivatives.



7 R^1 = BOC, R^2 = Me
8 R^1 = R^2 = H

9 R = H (Glutathione)
10 R = CH_2-CCH

Figure 3. Structure of *S*-propargyl-cysteine derivatives.

2. Results

In this model study, we investigated the coupling of 2-azido ethyl glycosides of different anomeric configurations. Three pyranosides derivatives of gluco, manno, and galacto configuration were chosen as the sugar components. Compounds **1**, **3,** and **5** were easily prepared by boron trifluoride–etherate catalyzed glycosylation of the peracetylated sugars with bromoethanol, and subsequent bromine substitution by azide carried out in hot DMF. The three compounds of β-gluco configuration (**1**),[11] α-manno (**3**),[12] and β-galacto (**5**)[10k] were obtained in 65%, 65%, and 60% yield, respectively. They were quantitatively deprotected to compounds **2**, **4,** and **6,** respectively, in basic medium (Fig. 2).

On the other hand, several cysteine-containing peptide models were prepared by S-alkylation under different conditions. Boc-OMe-cysteine was reacted with propargyl bromide in DMF in the presence of cesium carbonate[6c] providing the protected cysteine derivative **7** in 55% yield. The latter was in turn deprotected by successive basic hydrolysis and acidic treatment to provide the known free amino-acid **8**[6b] in 66% yield.

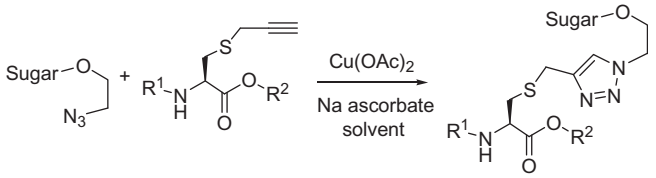
A direct method of thiol alkylation on the free peptide was investigated. To this end glutathione was chosen as a model of free tripeptide. Glutathione (**9**) was treated in aqueous basic solution (0.4 N $Ba(OH)_2$) followed by the addition of 1 equiv of freshly distilled propargyl bromide at room temperature. S-alkylation took place in 15 h and the *S*-propargyl-glutathione derivative **10** was obtained in 81% yield.[13] Proton NMR spectrum of the crude compound showed that an almost pure compound was formed bearing a single propargyl group. Only one signal corresponding to the propargylic proton was observed at 2.60 ppm. The chemical shifts observed for the methylene group of the propargyl at 3.29 ppm in proton NMR and at 18.9 ppm in carbon NMR established the formation of a *S*-propargyl appendage. Mass spectrum confirmed the structure (Fig. 3).

Click chemistry was then explored with the three protected sugars **1**, **3,** and **5** and the three *S*-propargyl derivatives **7**, **8,** and **10**. The reaction was performed in a 1:1 *tert*-butyl alcohol–water mixture using copper(II) acetate as the catalyst in the presence of sodium ascorbate as the reducing agent.[14] The results of these cycloadditions are summarized on Table 1. As seen from this Table, the reactions proceed under very mild conditions giving high yields of the expected 1,2,3-triazoles as the only products[15,16] (Fig. 4). Interestingly the reaction medium which became immediately light green on the addition of copper acetate to the mixture of the alkyne and the azide component turned blue within 1–2 h at which time the reaction was completed as seen from sugar consumption by TLC.

To test the efficiency of the ligation reaction between water soluble compounds, the click cycloaddition of the sugars **2**, **4,** and **6** with the tripeptide model glutathione **10** was investigated. In this case, water was used as the solvent. The reactions proceed equally well in short time. Treatment consisted of the removal of copper salts with a chelating resin and gel filtration. Compounds **20–22** were obtained in good yields.

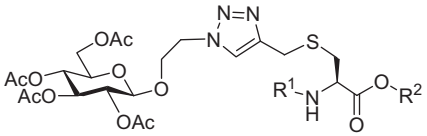
Finally, we explored the click ligation of a biologically significant peptide, that is, the adhesion peptide Arg-Gly-Asp-Cys (RGDC). Glycosylation of this peptide and its analogs has been

Table 1
Click ligation of azido-carbohydrate and *S*-propargyl amino-acids and peptides

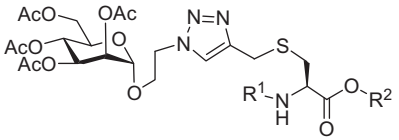


Entry	Azido component	Alkyne component	Product	Time (h)	Yield (%)
1	**1**	**7**	**11**	2[a]	73
2	**1**	**8**	**12**	2[a]	70
3	**1**	**10**	**17**	2[a]	83
4	**3**	**7**	**13**	2[a]	90
5	**3**	**8**	**14**	2[a]	75
6	**3**	**10**	**18**	2[a]	85
7	**5**	**7**	**15**	2[a]	73
8	**5**	**8**	**16**	1[a]	62
9	**5**	**10**	**19**	1[a]	60
10	**2**	**10**	**20**	1[b]	66
11	**4**	**10**	**21**	1[b]	62
12	**6**	**10**	**22**	1[b]	55
13	**6**	**24**	**25**	1[b]	52
14	**27**	**24**	**26**	1[b]	97

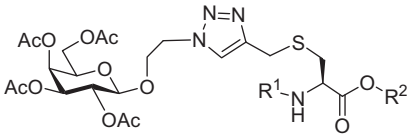
[a] The reaction was conducted in a 1:1 *tert*-butanol–water mixture at room temperature in the presence of 10 mol % of $Cu(OAc)_2$–H_2O and 20 mol % of sodium ascorbate.
[b] The reaction was conducted in water at room temperature in the presence of 10 mol % of $Cu(OAc)_2$–H_2O and 20 mol % of sodium ascorbate.



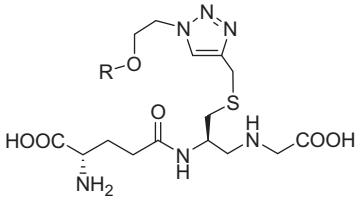
11 R^1 = BOC, R^2 = Me
12 R^1 = R^2 = H



13 R^1 = BOC, R^2 = Me
14 R^1 = R^2 = H

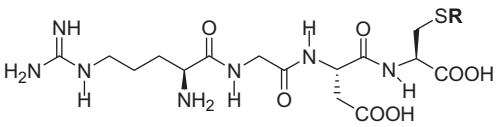


15 R^1 = BOC, R^2 = Me
16 R^1 = R^2 = H

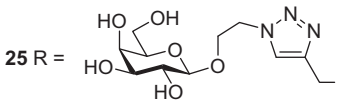


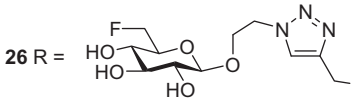
17 R = tetra-0-acetyl-β-D-glucopyranosyl
18 R = tetra-0-acetyl-α-D-mannopyranosyl
19 R = tetra-0-acetyl-β-D-galactopyranosyl
20 R = β-D-glucopyranosyl
21 R = α-D-mannopyranosyl
22 R = β-D-galactopyranosyl

Figure 4. Structure of glutathione–sugar adducts.



23 R = H (RGDC)
24 R = CH_2-CCH





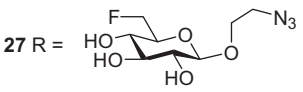


Figure 5. Structure of RGDC–sugar adducts.

explored and proved useful to target tumor cells and for imaging of tumor angiogenesis.[17] We chose to use two free carbohydrates, the galactoside **6** and the 6-fluoro-6-deoxy glucoside **27**. Commercially available RGDC (**23**) was first propargylated as above by treatment with propargyl bromide in water in the presence of a base for 1 h. A cleaner compound was obtained when using ammonia as a base.[7] NMR of the crude **24** showed almost one species in up to 85% purity. Only one signal corresponding to the propargylic proton was observed at 2.63 ppm. The crude compound **24** was then reacted with the appropriate azido compound in water. Although the reaction was difficult to monitor it appeared from color changing that the reaction was completed within 1–2 h. Copper ions' removal and gel filtration gave the two cycloadducts **25** and **26** in 52% and 67% yield, respectively[18] (Fig. 5 and Table 1).

From these results it appears that *S*-propargyl-cysteine derivatives including highly functional tri and tetrapeptides are easily available in a highly regiospecific manner by the treatment of free peptides with propargyl bromide in basic medium. This simple derivatization can be performed in the presence of carboxylic, amino, and guanidino groups. 2-Azido ethyl glycosides react efficiently with these alkynylated peptides in a click Huisgen reaction, whatever the sugar configurations (α-gluco, β-manno and α-galacto) and the anomeric configuration. The reaction proceeds well in water in short times and high yields and tolerates the presence of a fluorine atom on the sugar.

In conclusion, the facile propargylation of cysteine residues and the excellent reactivity of the resulting *S*-propargyl ether with azido ethyl glycosides provide an easy and fast ligation method of sugars with cysteine-containing peptides. We are currently exploring the use of this method on more complex biologically significant peptides with the aim of constructing labeled glycopeptides from labeled sugars.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.10.021.

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13. *Selected data for* **10**: white solid, $[\alpha]_D^{25}$ −22.9 (*c* 1.5, H_2O); ^1H NMR (D_2O, 400 MHz): δ 2.07 (dd, 2H, *J* = 7.5 Hz, *J* = 15.0 Hz, Hβ Glu), 2.45 (m, 2H, H-γ Glu), 2.60 (dd, 1H, *J* = 2.5 Hz, H-alkyne), 2.92 (dd, 1H, *J* = 9.0, *J* = 14.5 Hz, H-β Cys), 3.18 (dd, 1H, *J* = 5.0 Hz, H-β′ Cys), 3.29 (m, 2H, H-γ Cys), 3.70 (m, 3H, H-α Glu, H-α Gly), 4.65 (dd, 1H, H-α Cys); ^{13}C NMR (D_2O, 100 MHz): δ 18.9 (C-γ Cys), 26.2 (C-β Glu), 31.4 (C-γ Glu), 32.7 (C-β Cys), 43.4 (C-α Gly), 52.8 (C-α Cys), 54.1 (C-α Glu), 72.5 (CH alkyne), 80.3 (C alkyne), 171.8 (CO), 173.9 (CO), 174.9 (CO), 176.2 (CO); MS (ESI) 368.2 $[M+Na]^+$.
14. *General procedure for S-propargyl peptides and free sugars adduct formation*: To a solution of crude *S*-propargyl derivative (0.2 mmol), of the azido sugar (0.2 mmol) and sodium ascorbate (8 mg, 0.04 mmol) in water (4 ml) was added, at room temperature a solution of copper(II) acetate (4 mg, 0.02 mmol) in water (0.5 ml). The solution turned immediately pale green. The reaction was stopped when the intense blue color reappeared. Chelex resin (500 mg) was then added to the blue solution and the suspension was stirred until the solution became colorless. The resin was filtered off and the resulting solution was freeze-dried. Purification was achieved by LH20 gel filtration. Elution with water provided pure triazole.
15. It must be noted that the 1,4 triazole is the major product but is often contaminated by less than 10% of another yet unknown compound which is not 1,5 triazole as seen from proton and carbon NMR spectra. The structure of the latter was also established on the basis of carbon NMR (see Ref. 10h).
16. *Selected data of neoglycopeptides prepared from glutathione: S-[[1-[2-[β-D-glucopyranosyl)oxy]ethyl]-1H-1,2,3-triazol-4-yl]methyl]-L-glutathione* **(20)**: white solid, $[\alpha]_D^{25}$ −20.4 (*c* 0.4, H_2O); ^1H NMR (D_2O, 400 MHz): δ 2.19 (m, 2H), 2.55 (m, 2H), 2.88 (dd, 1H, *J* = 8 Hz, *J* = 14 Hz), 3.08 (dd, 1H, *J* = 5 Hz, *J* = 14 Hz), 3.27(dd, 1H, *J* = 8.5 Hz), 3.36–3.55 (m, 3H), 3.69 (dd, 1H, *J* = 6 Hz, *J* = 13 Hz), 3.75–3.95 (m, 6H), 4.15 (m, 1H), 4.33 (m, 2H), 4.45 (d, 1H, *J* = 8 Hz, H-1), 4.56 (dd, 1H, *J* = 4.8 Hz, *J* = 9 Hz), 4.72 (dd, 1H, *J* = 5 Hz, Hα Glu), 8.08 (s, 1H, C═CH); ^{13}C NMR (D_2O, 100 MHz): δ 25.6 (CH_2), 26.5 (CH_2), 31.7 (CH_2), 33.1 (CH_2), 43.7 (CH_2 Gly), 50.7 (CH_2), 53.1 (CH Cys), 54.4 (CH Glu), 61.0 (CH_2-6), 68.4 (CH_2), 69.9 (CH-4), 73.3 (CH-5), 75.9 (CH-2), 76.2 (CH-3), 102.7 (CH anomer), 125.1 (CH triazole), 145.1 (C═C), 172.2 (CO), 174.3 (CO), 175.2 (CO), 176.6 (CO); MS (HR-ESI) calcd for $C_{21}H_{33}N_6Na_2O_{12}S$ $[M-H+2Na]^+$ 639.1673, found: 639.1672. *S-[[1-[2-[(β-D-Mannopyranosyl)oxy]ethyl]-1H-1,2,3-triazol-4-yl]methyl]-L-glutathione* (**21**): white solid, $[\alpha]_D^{25}$ −3.5 (*c* 0.4, H_2O); ^1H NMR (D_2O, 400 MHz): δ 2.13 (m, 2H, Hβ Glu), 2.50 (m, 2H, Hγ Glu), 2.85 (m, 1H, Hβ Cys), 3.05 (m, 2H), 3.59 (dd, 1H, *J* = 9.5 Hz, *J* = 9.5 Hz), 3.61–4.0 (m, H), 4.10 (m, 1H), 4.54 (dd, 1H, *J* = 5 Hz, *J* = 9 Hz, Hα Cys), 4.67 (m, 2H), 8.03 (s, 1H, C═CH); ^{13}C NMR (D_2O, 100 MHz): δ 25.6 (CH_2), 27.1 (CH_2), 31.8 (CH_2), 33.1 (CH_2), 43.7 (CH_2 Gly), 50.5 (CH_2), 53.1 (CH Cys), 54.6 (CH Glu), 61.0 (CH_2-6), 65.8 (CH_2), 66.7 (C-4), 70.2 (C-5), 70.7 (C-2), 73.1 (C-3), 99.9 (CH anomer), 125.0 (CH triazole), 145.3 (C═C), 172.2 (CO), 175.3 (CO), 175.4 (CO), 176.6 (CO); MS (HR-ESI) calcd for $C_{21}H_{33}N_6O_{12}S$ $[M-H]^-$ 593.1883, found: 593.1905. *S-[[1-[2-[(β-D-Galactopyranosyl)oxy]ethyl]-1H-1,2,3-triazol-4-yl]methyl]-L-glutathione* (**22**): white solid, $[\alpha]_D^{25}$ −14.2 (*c* 0.6, H_2O); ^1H NMR (D_2O, 400 MHz): δ 2.13 (m, 2H), 2.50 (m, 2H), 2.84 (dd, 1H, *J* = 8 Hz, *J* = 14 Hz), 3.05 (dd, 1H, *J* = 5 Hz, *J* = 14 Hz), 3.48 (dd, 1H, *J* = 8 Hz, *J* = 9 Hz), 3.60–3.8 (m, 8H), 3.90 (m, 3H), 4.11 (m, 1H), 4.28 (m, 1H), 4.35 (d, 1H, *J* = 8 Hz, H-1), 4.51 (dd, 1H, *J* = 4.8 Hz, *J* = 9 Hz), 4.72 (dd, 1H, *J* = 5 Hz), 8.05 (s, 1H, C═CH); ^{13}C NMR (D_2O, 100 MHz): δ 25.6 (CH_2), 26.9 (CH_2), 31.8 (CH_2), 33.1 (CH_2), 43.7 (CH_2 Gly), 50.7 (CH_2), 53.1 (CH Cys), 54.6 (CH Glu), 61.3 (CH_2-6), 68.4 (CH_2), 68.9 (CH), 70.9 (CH), 73.0 (CH), 75.5 (CH_2), 103.4 (CH anomer), 125.2 (CH), 145.1 (C═C), 172.2 (CO), 175.0 (CO), 175.3 (CO), 176.6 (CO); MS (HR-ESI) calcd for $C_{21}H_{32}N_6O_{12}S$ $[M-2H]^{2-}$ 296.0905, found: 296.0920.
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18. *Selected data of neoglycopeptides prepared from RGDC*: *Arg-Gly-Asp-Cys-S-[[1-[2-[β-D-galactopyranosyl)oxy]ethyl]-1H-1,2,3-triazol-4-yl]methyl]* (**25**): white solid, $[\alpha]_D^{25}$ −14.8 (*c* 0.4, H_2O); ^1H NMR (D_2O, 400 MHz): δ 1.64 (m, 2H), 1.73 (m, 2H), 2.61 (dd, 1H, *J* = 8 Hz, *J* = 16 Hz), 2.74 (dd, 1H, *J* = 4 Hz, *J* = 16 Hz), 2.86 (dd, 1H, *J* = 5 Hz, *J* = 7 Hz), 2.98 (dd, 1H, *J* = 4.5 Hz, *J* = 14 Hz), 3.18–3.29 (m, 3H), 3.48 (dd, 1H, *J* = 8.5 Hz, *J* = 8.5 Hz), 3.56–3.80 (m, 2H), 3.84–3.98 (m, 5 H), 4.06–4.15 (m, 3H), 4.26–4.38 (m, 4H), 4.64–4.70 (m, 2H), 8.04 (s, 1H, C═CH); ^{13}C NMR (D_2O, 100 MHz): δ 24.3 (CH_2), 25.6 (CH_2), 30.9 (CH_2), 33.7 (CH_2), 38.6 (CH_2), 41.1 (CH_2), 42.7 (CH_2), 50.7 (CH_2), 51.8 (CH), 54.2 (CH), 54.7 (CH), 61.3 (CH_2-6), 68.3 (CH_2), 68.9 (CH), 70.9 (CH), 73.0 (CH), 75.5 (CH), 103.4 (CH anomer), 125.2 (CH), 145.2 (C═C), 157.1 (C═NH), 171.4 (CO), 172.9 (CO), 176.7 (CO), 178.0 (CO), 178.3 (CO); MS (HR-ESI) calcd for $C_{26}H_{44}N_{10}NaO_{13}S$ $[M+Na]^+$ 759.2708, found: 759.2703. *Arg-Gly-Asp-Cys-S-[[1-[2-[6-deoxy-6-fluoro-β-D-glucopyranosyl)oxy]ethyl]-1H-1,2,3-triazol-4-yl]methyl]* (**26**): white solid, $[\alpha]_D^{25}$ −25.1 (*c* 0.3, H_2O); ^1H NMR (D_2O, 400 MHz): δ 1.7 (m, 2H), 1.96 (m, 2H), 2.61 (dd, 1H, *J* = 8 Hz, *J* = 16 Hz), 2.74 (dd, 1H, *J* = 4 Hz, *J* = 16 Hz), 2.86 (dd, 1H, *J* = 5 Hz, *J* = 7 Hz), 2.98 (dd, 1H, *J* = 4.5 Hz, *J* = 14 Hz), 3.18–3.29 (m, 3H), 3.43–3.60 (m, 3H), 3.86 (s, 2H), 3.97 (d, 1H, *J* = 10 Hz), 4.06–4.28 (m, 3H), 4.24–4.38 (m, 3H), 4.44 (d, 1H, *J* = 5 Hz), 4.6–4.74 (m, 3H), 8.01 (s, 1H, C═CH); ^{13}C NMR (D_2O, 100 MHz): δ 23.7 (CH_2), 25.6 (CH_2), 28.3 (CH_2), 33.7 (CH_2), 38.5 (CH_2), 40.7 (CH_2), 42.8 (CH_2), 50.7 (CH_2), 52.0 (CH_2), 53.2 (CH), 54.6 (CH), 68.5 (CH), 73.2 (CH_2), 74.6 (CH), 75.7 (CH), 81.4 (CH), 83.0 (CH), 102.9 (CH anomer), 125.2 (CH), 145.3 (C), 157.1 (C═N), 170.5 (CO), 171.1 (CO), 172.9 (CO), 176.7 (CO), 178.2 (CO); ^{19}F NMR (D_2O, 235 MHz): δ −235 (ddd, *J* = 46 Hz, *J* = 24 Hz); MS (HR-ESI) calcd for $C_{26}H_{42}FN_{10}O_{12}S$ $[M-H]^-$ 737.2688, found: 737.2710.