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Application of the four-component Ugi condensation for the preparation of sulfated glycoconjugate libraries

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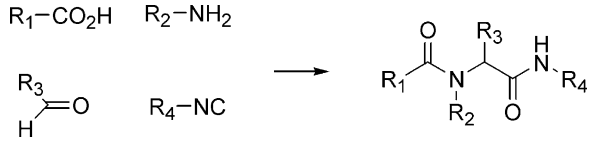
Abstract—A focused library of novel, sulfated glycoconjugates was synthesized by utilizing carbohydrate-derived blocks in the four-component Ugi condensation. Library members comprise a sulfated monosaccharide linked by various spacers to either an aromatic or monosulfated moiety, or a second sulfated monosaccharide. The affinities of these heparan sulfate (HS) mimetics for the HS-binding fibroblast growth factors FGF-1 and FGF-2 were measured via a surface plasmon resonance solution affinity assay.

Heparin and heparan sulfate (HS) are two important members of the glycosaminoglycan (GAG) family of linear, polyanionic polysaccharides that consists of repeating disaccharide units of uronic acid-(1→4)-D-glucosamine.[1,2] Variable patterns of substitution of each disaccharide unit with *N*-sulfo, *O*-sulfo and *N*-acetyl groups provide a large number of complex sequences that encode a high density of biochemical information. While such diverse structures contribute to their wide range of biological activities,[3,4] they also limit their clinical usefulness as drugs because of unwanted side effects. It is therefore desirable to develop HS mimetics that could specifically target HS-binding proteins.

During the previous decade, multicomponent reactions such as the four-component Ugi condensation[5] (Scheme 1) have attracted much attention in drug discovery and lead optimization,[6–8] including the glycomics area,[9–14] because of their synthetic potential for the generation of molecular diversity and applications in combinatorial chemistry. In this communication we report the first examples of the synthesis of sulfated glycoconjugates based on the Ugi reaction and their affinities for the HS-binding fibroblast growth factors 1 and 2 [FGF-(1,2)].



Scheme 1. Generic representation of the four-component Ugi condensation.

The choice of FGF-(1,2) as targets is based on their important roles in a wide variety of physiological processes related to cell proliferation, differentiation and migration, as well as disease processes such as tumour angiogenesis.[15–17] These FGFs are also among the most-studied HS-binding proteins with a number of X-ray crystal structures published, including FGF in complex with heparin-derived oligosaccharides,[18,19] and FGF in ternary complex with its receptor (FGFR) and a heparin-derived oligosaccharide.[20,21] FGF-(1,2) are attractive targets for antiangiogenic and antitumour drug design[22] and a number of small molecules have been shown to inhibit FGF-mediated mitogenic activity by binding to FGF-(1,2) and blocking the formation of the heparin:FGF:FGFR complex.[23–31]

A focused library of HS mimetics was designed with at least one component derived from a persulfated monosaccharide (Fig. 1) to target the positively charged

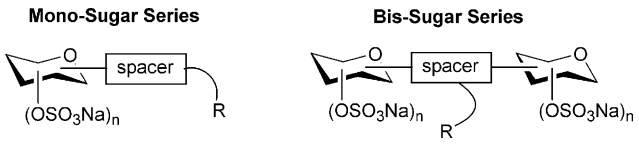


Figure 1. Design of the library of sulfated glycoconjugates. R = an aromatic, an aliphatic or a negatively charged group (e.g., sulfo group).

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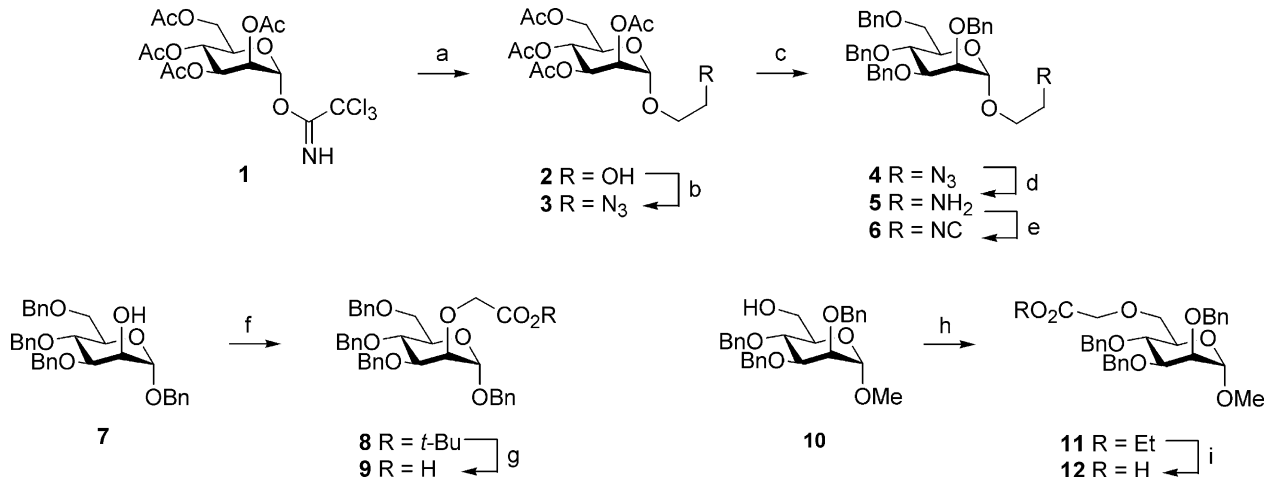


Scheme 2. Reagents and conditions: (a) ethylene glycol, MS 3 Å, CH_2Cl_2, $BF_3 \cdot Et_2O$, −20 °C, 1.5 h; (b) (i) MsCl, Et_3N, CH_2Cl_2, 0–25 °C, 3 h, (ii) NaN_3, DMF, N_2, 50 °C, 2 h, 45% (three steps); (c) (i) NaOMe, MeOH, 25 °C, 1.5 h, (ii) NaH, BnBr, DMF, 25 °C, 2 h, 58% (two steps); (d) PPh_3, THF–H_2O (7:1), 25 °C, o/n, 96%, or $NaBH_4$, $NiCl_2$, MeOH–THF (3:2), 0–25° C, 1 h, 89%; (e) (i) Ac_2O, HCO_2H, 25 °C, 3 h, 92%, (ii) $POCl_3$, Et_3N, CH_2Cl_2, 0 °C, 1 h, 92%; (f) NaH, DMF, $BrCH_2CO_2Bu^t$, 25 °C, 4 h; (g) 5% TFA in CH_2Cl_2, 25 °C, 0.5 h, 90% (two steps); (h) NaH, DMF, $BrCH_2CO_2Et$, 25 °C, 19 h, 51%; (i) NaOH, THF–MeOH (1:1), 70 °C, 3 h, 70%.

residues on the surface of the HS binding site of FGF-(1,2).[18,19] The monosugar series of compounds contains a single, persulfated monosaccharide linked via a spacer (formed by the Ugi reaction) to either an aromatic, an aliphatic or a negatively charged functional group. These structures are similar in size to a disaccharide and are useful for studying the contributions of hydrophobic or additional ionic motifs within the HS binding site. The bis-sugar series of compounds contains two persulfated monosaccharide units linked by a spacer. These structures are designed to mimic sulfated oligosaccharides with the central sugar units replaced by a spacer, and be suitable for investigating the spatial relationship between the two negatively charged monosaccharide units. In addition, due to restricted rotation about the amide bonds, the conformational flexibility of these types of structures is greatly reduced and may offer a restricted presentation of sulfo groups.[13,14] Recently a large, random combinatorial library was prepared via the Ugi reaction and screened for inhibition of heparin binding to FGF-2.[25] As expected, the identified hits all contained some negatively charged functional groups.

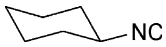
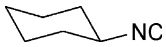
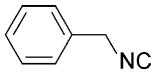
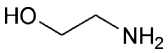
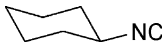
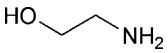
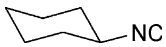
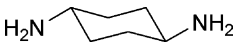
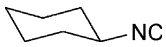
To illustrate the potential diversity offered by this type of library, monosaccharide building blocks were prepared containing three of the four types of functional groups required for the Ugi condensation (amine, isocyanide and carboxylic acid) at various positions around the hexopyranose ring. To simplify the characterization of the products, a symmetrical carbonyl component, formaldehyde,[†] was generally used in library production. The preparation of the carbohydrate building blocks is outlined in Scheme 2. Glycosylation of the trichloroacetimidate[32] **1** with ethylene glycol followed by standard functional group transformations and protecting group manipulation provided the amino block **5** in 25% overall yield. This was converted into the isocyanide block **6** in high yield via formylation and dehydration. The acid blocks **9** and **12** were prepared via esters (*tert*-butyl or ethyl) by alkylation of the corresponding alcohols **7**[33] and **10**,[34] followed by hydrolysis.[‡] The commercially available D-glucuronic acid was also used as an acid block.

The components used for the Ugi reaction are outlined in Table 1. The monosugar series (Table 1, entries 1–6 and 10) was constructed by using a carbohydrate acid block (D-glucuronic acid or **12**) with commercially available amine, isocyanide and carbonyl components.[35] One example using the carbohydrate isocyanide block **6** (entry 10) was also prepared. The bis-sugar series was prepared by Ugi reaction incorporating two carbohydrate blocks (entries 7 and 9), or by one incorporating a single carbohydrate block and a bis-amine (*trans*-1,4-diaminocyclohexane, entry 8) or bis-acid (3,3-dimethylglutaric acid, entry 11). The products, isolated in good to moderate yield (36–72%), were deprotected (NaOMe/MeOH, entries 1–4; or H_2/Pd/C, entries 5–11) and sulfonated[36] to provide compounds **14**–**24** (Fig. 2).[37] The partially sulfated product **13** was separable from its fully sulfated counterpart **14** by flash chromatography (MeCN–Et_3N–H_2O, 110:2:11). Interestingly, the sulfated products in the D-glucuronic acid series (**13**–**17**) were isolated exclusively as α-anomers, as determined by ^1H NMR spectroscopy (typically $J_{1,2} \sim 3.5$ Hz).

[†] Formaldehyde was added from a stock solution in MeOH (2 M) made from the corresponding commercially available aqueous solution (36.5–38.0% w/w, aqueous solution stabilized with 10% MeOH).

[‡] The corresponding *tert*-butyl ester of **10** was susceptible to polymerization upon acid catalyzed deprotection. The ethyl ester **11** was therefore used in this case.

Table 1. Components used for the Ugi reaction, sulfated products (shown in Fig. 2), and dissociation constants measured for sulfated products binding to FGF-(1,2)

Entry	Components for Ugi reaction				Yield (%)[a]	Sulfated product	K_d (μM)[b]	
	Acid	Amine	Aldehyde	Isocyanide			FGF-1	FGF-2
1	D-GlcA	$BnNH_2$	HCHO		66	**13**[c]	587 ± 246	1160 ± 190
						14	122 ± 13	254 ± 12
2	D-GlcA	$BnNH_2$	PhCHO		46	**15**	62.5 ± 1.3	>800[d]
3	D-GlcA	$BnNH_2$	HCHO		36	**16**	104 ± 52.3	206 ± 11
4	D-GlcA		HCHO		47	**17**	2340 ± 690	>800[d]
5	**12**	$BnNH_2$	HCHO		72	**18**	260 ± 4	200 ± 36
6	**12**		HCHO		66	**19**	296 ± 4	551 ± 96
7	**12**	$BnNH_2$	HCHO	**6**	64	**20**	4.84 ± 0.16	16.0 ± 3.0
8	**12**		HCHO		43	**21**	32.6 ± 23.6	27.9 ± 1.5
9	**9**	$BnNH_2$	HCHO	**6**	36	**22**	6.36 ± 1.2	85.0 ± 13.0
10	CH_3CO_2H	$BnNH_2$	HCHO	**6**	67	**23**	34.3 ± 0.2	287 ± 27
11		**5**	HCHO		67	**24**	10.0 ± 4.0	34.0 ± 13.0

[a] Unoptimized percentage yields for initial Ugi reactions (including peracetylation, if required).
[b] The average and standard deviation of at least two independent measurements.
[c] Undersulfated product isolated by chromatography.
[d] No binding observed up to this concentration.

The binding affinities of the sulfated glycoconjugates **13**–**24** for FGF-(1,2) were measured using a surface plasmon resonance (SPR) solution affinity assay[38] (Table 1). The principle of the SPR assay is that a solution, at equilibrium, of the growth factor and a ligand is passed over a sensor chip containing immobilized heparin. As the unbound growth factor binds to the heparin, an increase in the SPR response is detected from which the concentration can be determined. The dissociation constant, K_d, can be calculated from the decrease in the free growth factor concentration as a function of ligand concentration.

The measured K_d values range over three orders of magnitude from 10^{-3} to 10^{-6} M. Although the affinities are not as strong as for heparin/HS or the phosphosulfomannan PI-88,[38] some of the compounds show promising affinities, particularly the monosugar compounds, which have relatively few sulfo groups. The small size of the library does not allow a complete SAR analysis; however, some trends are evident. The number of sulfo groups is clearly important, as indicated by the decrease in affinity upon loss of a single sulfo group (**13** vs **14**), and the generally higher affinity seen in the bissugar series (**20**–**22**, **24**). The introduction of additional charge by replacement of an aromatic group with a sulfo group leads to a significant decrease in affinity for the D-glucuronic acid series (**14** vs **17**) and no change for the D-mannose series (**18** vs **19**), indicating that the spatial arrangement of the sulfo groups is also important for good binding.

The potential for synthesizing larger, more diverse combinatorial libraries of this type is enormous given that (i) carbohydrate blocks can be used for any of the four Ugi components,[10] (ii) a large number of different carbohydrates are available and (iii) there is the ability to introduce the required functional groups at any position(s) around the hexopyranose ring via standard carbohydrate protecting group manipulations. In addition, it should be possible to limit both the number of sulfo groups and their placement in specific positions around the hexopyranose ring by using either orthogonally protected, or differentially sulfated carbohydrate Ugi blocks. These latter possibilities are currently under investigation.

In conclusion, a library of sulfated glycoconjugates as heparin/HS mimetics has been synthesized via the four-component Ugi condensation utilizing one or two monosaccharide-derived building blocks. These compounds show promising affinities for the HS binding,

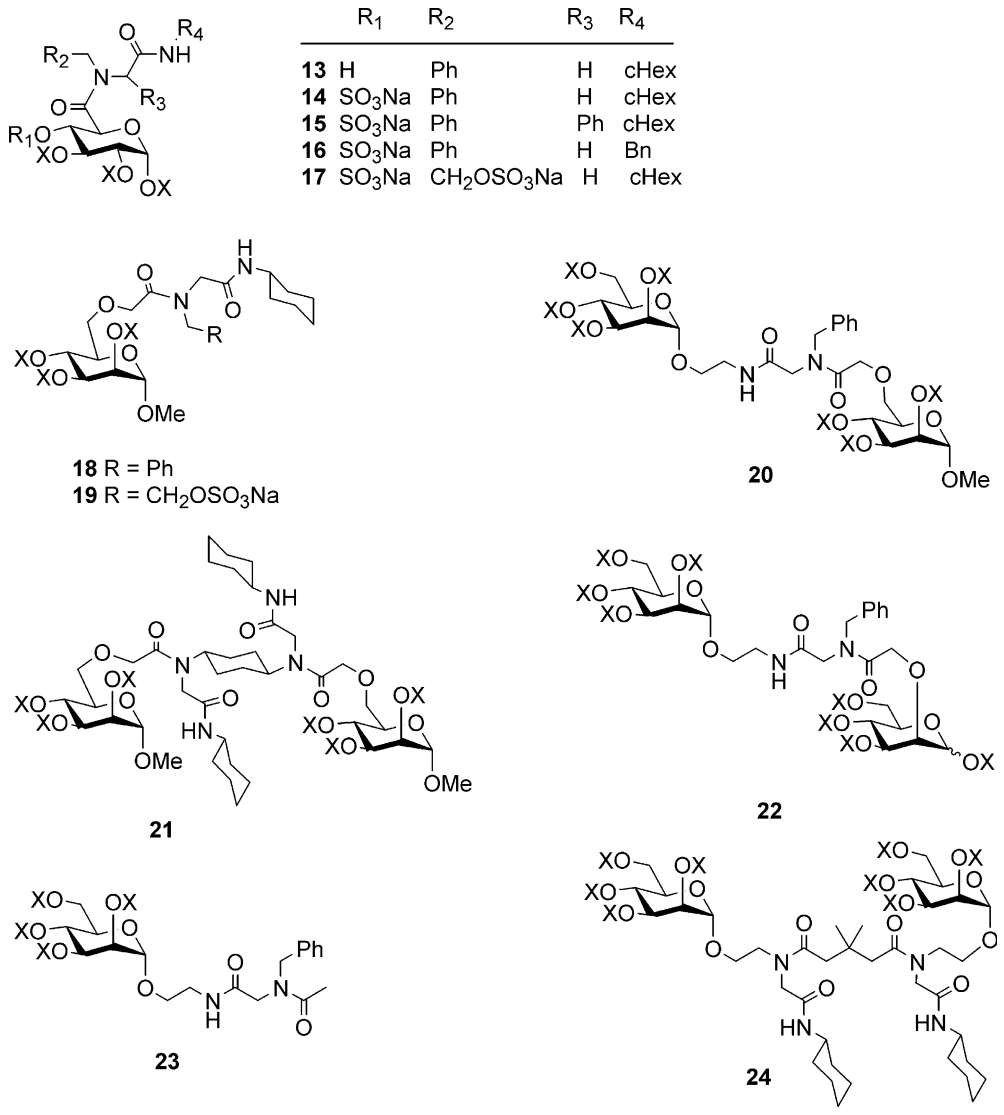


Figure 2. Structures of compounds evaluated for binding to FGF-(1,2) in the SPR solution affinity assay (dissociation constants given in Table 1). X = SO_3Na.

angiogenic growth factors FGF-(1,2). This strategy provides useful biological tools to study heparin/HS-binding proteins as well as information for refinement of ligands to achieve improved activity and selectivity. The use of unprotected components in the Ugi reaction such as D-glucuronic acid, greatly simplifies the synthetic procedures and represents a platform for the rapid assembly of such products by using inexpensive, readily available starting materials.

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35. *General procedure for the Ugi reaction*: Solutions in MeOH of the acid (1 equiv), amine (1 equiv), aldehyde (1 equiv) and isocyanide (1 equiv) were transferred sequentially, in that order, into a reaction vial (final concentration: 0.1–0.5 M). When D-glucuronic acid was the acid component, it was added as a solid. When a bis-acid or bis-amine was used, only 0.5 equiv of this component was added. The mixture was shaken at rt for 24 h and the reaction was monitored by TLC. The mixture was then evaporated in vacuo and the residue either directly purified by flash chromatography, or, for entries 1–4 in Table 1, subjected to standard acetylation conditions (Ac_2O/pyridine) prior to chromatography.
36. *General procedure for sulfonation*: The polyol was dissolved in anhydrous DMF (0.04 M) and sulfur trioxide pyridine complex (2 equiv per hydroxyl group) or sulfur trioxide trimethylamine complex (3 equiv per hydroxyl group) was added. The mixture was stirred overnight at 60 °C under an atmosphere of nitrogen, cooled to 0 °C and basified by the addition of 3 M NaOH solution until the pH∼9. The mixture was evaporated to dryness in vacuo and the residue was purified by size-exclusion chromatography on Bio-Gel P-2 or LH-20, eluting with 0.1 M NH_4HCO_3 or water, respectively. The pure product fractions identified by capillary electrophoresis[39] were pooled, lyophilized, passed through an ion exchange column (AG 50W-X8, Na^+ form, 1 × 18 cm, eluting with deionized water) and lyophilized again to provide the sulfated product as the sodium salt.
37. The purity of sulfated products (⩾96%) was determined by capillary electrophoresis.[39] All compounds gave satisfactory NMR and MS data. The NMR spectra were complex due to the presence of rotamers formed by restricted rotation about the amide bonds but could be simplified in some cases by the use of high temperature (some coalescence was observed) and assigned with the aid of gCOSY and gHSQC experiments. Representative data: **13**: ^{1}H NMR (D_2O, 400 MHz, 25 °C, internal ref.: acetone, δ 2.05): two rotamers in a molar ratio of 56:44. *Major rotamer*: δ 7.36–7.11 (m, 5H, Ph), 5.95 (d, $J_{1,2} = 3.4$ Hz, H1), 4.89 (d, $J_{4,5} = 9.6$ Hz, H5), 4.75, 4.69 (AB, $J_{A,B} = 16$ Hz, a-CH_2), 4.50 (dd, $J_{2,3} = J_{3,4} = 9.6$ Hz, H3), 4.31 (dd, H2), 4.00 (dd, H4), 3.87 (s, b-CH_2), 3.42–3.32 (m, 1H, cyclohexyl CH), 1.64–1.36, 1.20–0.92 (2m, 5H each, cyclohexyl CH_2); *minor rotamer*: δ 7.36–7.11 (m, 5H, Ph), 5.90 (d, $J_{1,2} = 3.2$ Hz, H1), 4.58 (d, $J_{4,5} = 9.8$ Hz, H5), 4.52 (s, 2H, c-CH_2), 4.48 (dd, $J_{2,3} = 10$, $J_{3,4} = 9.4$ Hz, H3), 4.32, 3.90 (AB, $J_{A,B} = 17.6$ Hz, d-CH_2), 4.28 (dd, H2), 4.04 (dd, H4), 3.42–3.32 (m, 1H, cyclohexyl CH), 1.64–1.36, 1.20–0.92 (2m, 5H each, cyclohexyl CH_2); ^{13}C NMR (D_2O, 100 MHz, internal ref.: acetone, δ 30.37): doubling-up of signals due to two rotamers: δ 170.0, 169.8, 168.9, 168.8 (2×C=O), 135.4, 135.2, 129.2, 129.2, 128.5, 128.3, 128.2, 128.1 (Ph), 95.6(7), 95.6(5) (C1), 77.8, 77.7 (C3), 73.8, 73.7 (C2), 70.7, 70.2 (C4), 69.2, 68.7 (C5), 52.9 (a-CH_2), 51.1 (c-CH_2), 50.3 (d-CH_2), 50.0 (b-CH_2), 49.2, 49.0 (cyclohexyl CH), 31.8(9), 31.8(6), 25.1, 25.0, 24.5, 24.4 (cyclohexyl CH_2); HRMS: m/z calcd for $C_{21}H_{28}N_2Na_3O_{16}S_3$: 729.0294; found: 729.0242 $[M + H]^+$. **14**: ^{1}H NMR: two rotamers in a molar ratio of 70:30. *Major rotamer*: δ 7.34–7.16 (m, 5H, Ph), 5.95 (d, $J_{1,2} = 3.5$ Hz, H1), 5.24 (d, $J_{4,5} = 9.6$ Hz, H5), 4.90, 4.47 (AB, $J_{A,B} = 15.6$ Hz, a-CH_2), 4.67–4.57 (overlapped with water, 1H, H3), 4.54 (dd, $J_{3,4} = 8.8$ Hz, H4), 4.39 (dd, $J_{2,3} = 9.8$ Hz, H2), 3.93, 3.75 (ABq, $J_{A,B} = 16.8$ Hz, b-CH_2), 3.30–3.20 (m, 1H, cyclohexyl CH), 1.65–1.35, 1.18–0.92 (2m, 5H each, cyclohexyl CH_2); *minor rotamer*: δ 7.34–7.16 (m, 5H, Ph), 5.91 (d, 1H, $J_{1,2} = 3.4$ Hz, H1), 4.77–4.72 (m, 2H, H5 and H3 or H4), 4.69, 4.22 (AB, $J_{A,B} = 15.2$ Hz, c-CH_2), 4.67–4.56 (overlapped with water, 1H, H3 or H4), 4.37 (dd, $J_{2,3} = 9.8$ Hz, H2), 4.26, 3.94 (AB, $J_{A,B} = 18.4$ Hz, d-CH_2), 3.36–3.26 (m, 1H, cyclohexyl CH), 1.65–1.35, 1.18–0.92 (2m, 5H each, cyclohexyl CH_2); ^{13}C NMR: *major rotamer*: δ 172.4, 171.7 (2×C=O), 137.2, 131.7, 131.6, 131.1 (Ph), 97.7 (C1), 78.1 (C4), 77.6 (C3), 76.4 (C2), 70.1 (C5), 55.8 (a-CH_2), 53.2 (b-CH_2), 51.8 (cyclohexyl CH), 34.2, 27.6, 27.1 (cylcohexyl CH_2); *minor rotamer*: 97.6 (C1), 78.2 (C4), 77.8 (C3), 76.3 (C2), 71.0 (C5), 53.3 (d-CH_2), 53.8 (c-CH_2), 52.1 (cyclohexyl CH), 34.3, 27.5, 27.1 (cylcohexyl-CH_2); HRMS: m/z

calcd for $C_{21}H_{27}N_2Na_4O_{19}S_4$: 830.9682; found: 830.9635 $[M+H]^+$.

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