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Calculated Conformations of Sialyl-Le^x- and Sialyl-Le^a-Lactones

Ulf Ellervik and Göran Magnusson*

Organic Chemistry 2, Chemical Center, The Lund Institute of Technology, University of Lund, Box 124, 221 00 Lund, Sweden

Abstract—The minimum energy conformations of the four sterically reasonable SLe^x and SLe^a lactones were calculated using the molecular mechanics force-field MM2(91). The tetrasaccharide lactone involving the 3- and 2-position of the Gal moiety was found to be more stable than the 3,4-lactone both for SLe^x and SLe^a.

Introduction

The sialyl Lewis x (SLe^x) and sialyl Lewis a (SLe^a) tetrasaccharides (Fig. 1) are terminal constituents of membrane-bound glycoproteins and glycolipids. They are well known blood-group determinants. Recent reports have shown that these saccharides have a number of important biological functions.[1] SLe^x and

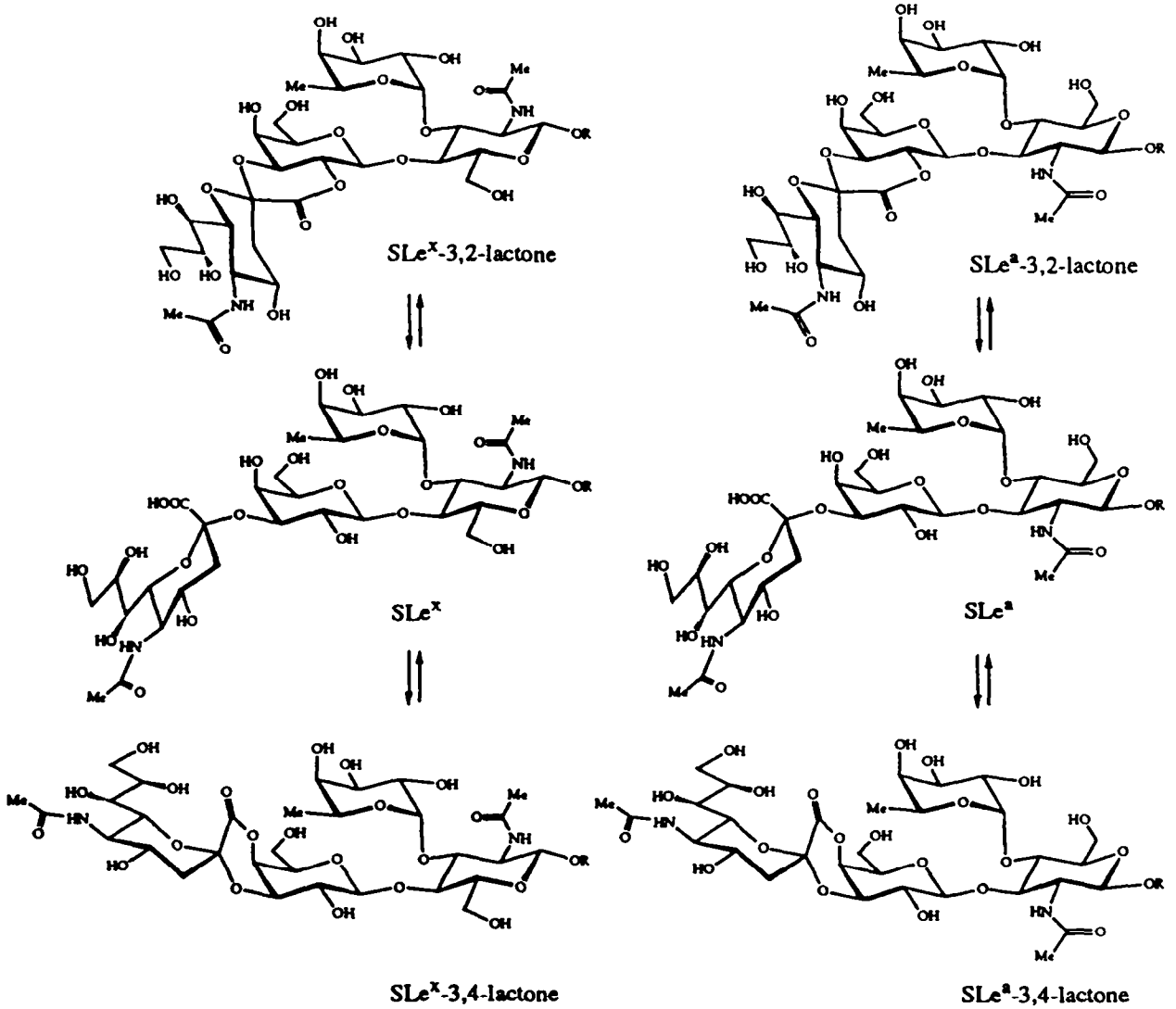

Figure 1. The four lactones corresponding to SLe^x and SLe^a.

SLe^a are tumor-associated antigens in connection with human pancreatic adenocarcinoma[2] and human gastrointestinal tumors[3] and have recently been identified as selectin binders of physiological importance for recruitment of leukocytes to inflammation sites.[4] The possibility of blocking selectin binding, using the SLe^x/SLe^a saccharides or their structural variants, has caused an upsurge of research activities concerning their organochemical synthesis[5] and biomedical applications.[6]

There is a striking conformational similarity between the SLe^x and SLe^a tetrasaccharides. Several research groups have found that *E*-selectin (ELAM-1) binds both saccharides with similar avidity and that the tetrasaccharide is the smallest oligosaccharide epitope that is recognized by the lectin; however, SLe^x analogs carrying a sulfate group instead of the NeuAc moiety showed similar biological activities.[7]

Sialic acid-containing saccharides (gangliosides) may undergo δ-lactone formation between the carboxyl group of the sialic acid moiety and a suitable hydroxyl group of its aglycon.[8] However, the presence on cells of ganglioside lactones has been subject to debate for many years. Positive indications have been obtained from sodium borodeuteride-reduction of ganglioside-containing cells followed by isolation of reduced gangliosides,[9] and also from isolation and structure determination of a ganglioside lactone from the human brain.[10] The existence of ganglioside lactones in cell samples has been inferred by detection with anti-ganglioside lactone antibodies.[11] However, safe conclusions could not be drawn since the antibodies cross-reacted to a certain extent with the non-lactonized form of the ganglioside.

We have recently obtained a number of antibodies via immunization with a synthetic GM_3-lactam BSA conjugate,[12] which is a synthetic analog of GM_3-ganglioside lactone, having similar overall shape.[13] The antibodies recognize GM_3-ganglioside lactone *in vitro* but not the normal, non-lactonized, GM_3-ganglioside.[12] Mouse melanoma cells, known to express high amounts of GM_3-ganglioside, were effectively stained by use of the anti-GM_3-lactam antibodies.[14] This is a strong indication that GM_3-ganglioside lactone is present on the surface of these cells.

Although SLe^x and SLe^a lactones have not been observed (or even suggested) in biological systems, they might well be of importance for fine tuning of selectin receptor function *in vivo*. Lactone formation was observed with a protected derivative during a total synthesis of SLe^x.[5a] The conformations of SLe^x and SLe^a in their non-lactonized forms, were recently published.[15] We now report the calculated conformations of the four possible SLe^x and SLe^a lactones (Fig. 1). The calculated conformational energies indicate a thermodynamic preference for lactone formation involving HO-2, as compared to HO-4, of the galactose unit. It should be noted that the calculations were performed with isolated molecules and that therefore the relative energies obtained may differ somewhat from that in an aqueous environment.

Results and Discussion

The structures of the four different SLe^x and SLe^a lactones (Fig. 1) were constructed using the MacMimic[16] program and the minimum energy conformations were calculated using the MacMimic/MM2(91) software package.[16] The program uses the unadulterated version of Allinger's MM2(91) force-field.[17] Initially, all the monosaccharide moieties were energy-minimized using starting conformations that avoid intramolecular hydrogen bonding. These structures were used for the construction of larger saccharides as discussed below.

The minimum energy conformations of the two NeuAc-Gal-lactones (GM_4-lactones) were calculated starting from lactone-ring chair and boat conformations, leading to low-energy twist and chair conformations as shown in Table 1. The starting conformations were estimated from Dreiding models and transferred to the MacMimic[16] program. One of the starting chairs and one of the boats of the 3,2-lactone (i.e. glycosidic bond to HO-3 of Gal and lactone ring formed with HO-2; see also Fig. 1) were transformed, during calculation, into two low-energy twist conformations of similar structure, whereas the remaining chair and boat ended up as chairs with considerably higher energy. The 3,4-lactone ended up as low-energy twist conformations regardless of the starting conformation. The lowest energy conformations of the two lactones were used in the construction of the SLe^x and SLe^a lactones, as discussed below.

Table 1. Low-energy conformations of the GM_4 3,2- and 3,4-lactones as calculated with the MM2(91)[16] force-field

Compound	Starting conform.[a]	Low-energy conform.[a]	Energy[b] (kJ/mol)
3,2-lactone	chair	twist	0.00
	chair	chair	15.7
	boat	twist	0.4
	boat	chair	26.9
3,4-lactone	chair	twist	2.1
	chair	twist	2.7
	boat	twist	2.1
	boat	twist	2.7

[a]Refers to the lactone ring.
[b]The conformation with the lowest energy was set to 0.00 kJ/mol.

The Le^x and Le^a trisaccharides were constructed from the energy-minimized monosaccharide moieties, using published data[15a] for the torsional angles of the anomeric bonds. The low energy conformations of the Le^x and Le^a trisaccharides were then calculated with the MacMimic/MM2(91) program package.[16]

The minimum energy conformations of the GM_4-lactones and Le^x and Le^a trisaccharides were then combined to give the four SLe^x and SLe^a lactones shown in Figure 1. Final energy minimizations gave the minimum energy conformations of the SLe^x and SLe^a tetrasaccharide lactones as shown in Table 2.

The conformational map for the NeuAc moiety of SLe^x shows four energy minima as depicted in Figure 2. The energy minima of SLe^x-3,2- and 3,4-lactones are rather close to energy minima of SLe^x, suggesting that lactone formation might be a rather facile process. The energy map is similar to that reported by Ichikawa *et al.*,[15a] although there are discrepancies in the positions of the global energy minima.

All lactone rings have a twist-boat conformation at energy minimum. The energy difference (Table 2) between the 3,2- and 3,4-lactones of SLe^x (or SLe^a) is greater than the difference between lactones of the same type (e.g. the 3,2-lactones of SLe^x and SLe^a).

The energy difference is larger for the tetrasaccharide lactones than for the GM_4-lactones. This indicates that the tetrasaccharides are rather rigid structures with little opportunity for internal movement, which is corroborated by conformational analysis based on NMR and molecular mechanics calculations.[15] For example, short distances were observed between H-2 (Gal) and H-5 (Fuc) in SLe^x.

Both 3,2- and 3,4-lactones have been observed for a synthetic intermediate (carrying protecting groups) en route to SLe^x.[5a] The ratio between 3,2- and 3,4-lactones was 77/23, which is close to the Boltzman distribution

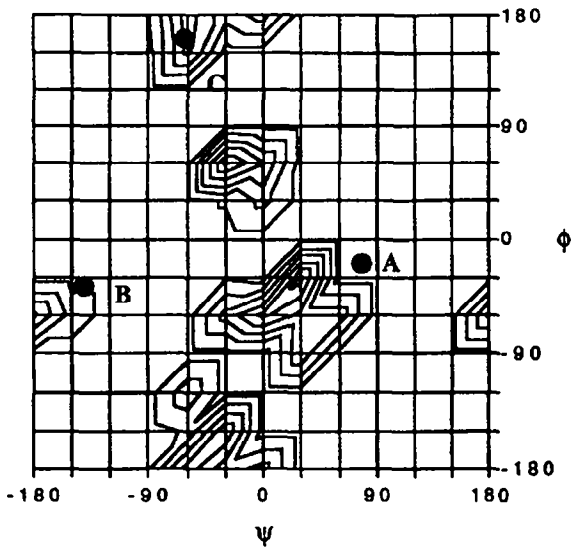

Figure 2. The NeuAc anomeric conformational space of SLe^x. The ϕ and Ψ angles are as defined in the Experimental section. Energy difference between equi-energetic curves is 2.1 kJ/mol; energies larger than 12.6 kJ/mol above the global minimum (close to A) are not shown. Black dots at A: Φ,Ψ-angles of SLe^x-3,2-lactone; B: Φ,Ψ-angles of SLe^x-3,4-lactone; C: Φ,Ψ-angles of SLe^x as reported in Ref. 15a and Table 2.

ratio of 82/18, calculated from the energy difference between (unprotected) SLe^x-3,2- and 3,4-lactones (Table 2). Thus, in all cases observed or calculated to date, 3,2-lactones seem to be somewhat more stable than 3,4-lactones.

The structural difference between the 3,2- and 3,4-lactones of SLe^x (and SLe^a) is depicted by RMS-fitting of the ring atoms of Gal, Fuc, and GlcNAc residues in the tetrasaccharides (Fig. 3). The corresponding trisaccharide moieties are practically overlapping (RMS = 0.04 and 0.03 Å, respectively), whereas the sialic acid and lactone rings occupy very different conformational space, in turn different from that of the non-lactonized parent saccharides (*c.f.* Fig. 5) . This means that SLe^x

Table 2. Calculated [MM2(91)[16]] energies and torsional anomeric bond angles for the minimum energy conformations of SLe^x, SLe^a and the four SLe^x and SLe^a lactones

Compound	Energy[a] (kJ/mol)	Angle (deg)					
		NeuAcα		Galβ		Fucα	
		Φ	Ψ	Φ	Ψ	Φ	Ψ
SLe^x-3,2-lactone	0.00	-19	86	48	11	33	27
SLe^x-3,4-lactone	3.9	-32	-138	50	9	33	27
SLe^x (global min.)[b]		-36	35	43	11	28	31
SLe^x (ref. 15a)		167	-63	48	15	22	30
SLe^a-3,2-lactone	0.6	-18	86	36	20	43	21
SLe^a-3,4-lactone	7.6	-32	-138	37	20	42	21
SLe^a (ref. 15a)		-179	-62	34	19	40	25

[a]The conformation with the lowest energy was set to 0.00 kJ/mol.
[b]The NeuAcα (Φ,Ψ) conformational space was searched in 30 ° increments, using the dihedral driver option of the MacMimic/MM2(91)[16] program; the resulting lowest energy conformation was then energy minimized without any structural constraints.

and SLe^a (as well as other sialic acid-containing saccharides) have the option of tuning their structures as a response to the various informational demands put upon these important biological receptor structures.

The SLe^x- and SLe^a-3,2-lactones (and 3,4-lactones) have very similar, albeit not identical shapes (Fig. 4; RMS = 0.21 and 0.25 Å, respectively), in contrast to the structural dissimilarity between the 3,2- and 3,4-lactones (Fig. 3). The RMS fitting was performed using all ring atoms of the NeuAc, lactone, Gal, Fuc, and GlcNAc residues. The structural similarity (but not identity) of the SLe^x- and SLe^a-3,2-lactones (and 3,4-lactones) provides an added opportunity to fine-tune the overall conformations by simply changing the positions where Gal and Fuc bind to the GlcNAc moiety.

The space-filling models of the SLe^x and SLe^a lactones (Fig. 5) show a conspicuous shallow hydrophilic groove across one side of the molecule, providing ample possibility for hydrogen bonding with, for example, a receptor protein. The remaining surfaces are largely hydrophobic. These saccharides therefore fit well into what seems to be a general pattern for receptor-active saccharides, namely large hydrophobic surface patches surrounding hydrophilic ridges or grooves with potential for hydrogen bonding.[18] The space-filling model of SLe^x is rather similar to that of SLe^x-3,2-lactone, reflecting the transition from the global energy minimum (close to ϕ/Ψ, -30°/30°) to A in Figure 2.

Experimental

Molecular mechanics calculations were performed using the unadulterated Allinger force-field,[17] which is available for Macintosh computers as the MacMimic/MM2(91)[16] program package. The standard force-field includes treatment of the O–C–O anomeric effect.[19] Calculations were performed with a dielectric constant setting of 80. The anomeric torsional angles are defined as Φ: H_1–C_1–O_1–$C_{x'}$ (for NeuAc, C_1–C_2–O_2–$C_{x'}$) and Ψ: C_1–O_1–$C_{x'}$–$H_{x'}$.

Monosaccharide structures were constructed with the MacMimic program and low-energy conformations were calculated. Care was taken to avoid intramolecular hydrogen bonding by orienting hydroxyl groups in suitable starting positions.

The di- and trisaccharide fragments were constructed from the energy-minimized monosaccharides. Starting anomeric torsional angles were chosen from literature data[15a] and low-energy conformations were calculated. Finally, the di- and trisaccharides were combined to give the four lactones SLe^x-3,2-lactone, SLe^x-3,4-lactone, SLe^a-3,2-lactone, and SLe^a-3,4-lactone (Fig. 1). The conformational energies were calculated (Table 2) and the low-energy conformations are depicted in Figures 3 and 4. A calculation was terminated when the energy difference between two consecutive conformers was < 0.00033N kJ/mol (N = number of atoms in the molecule).

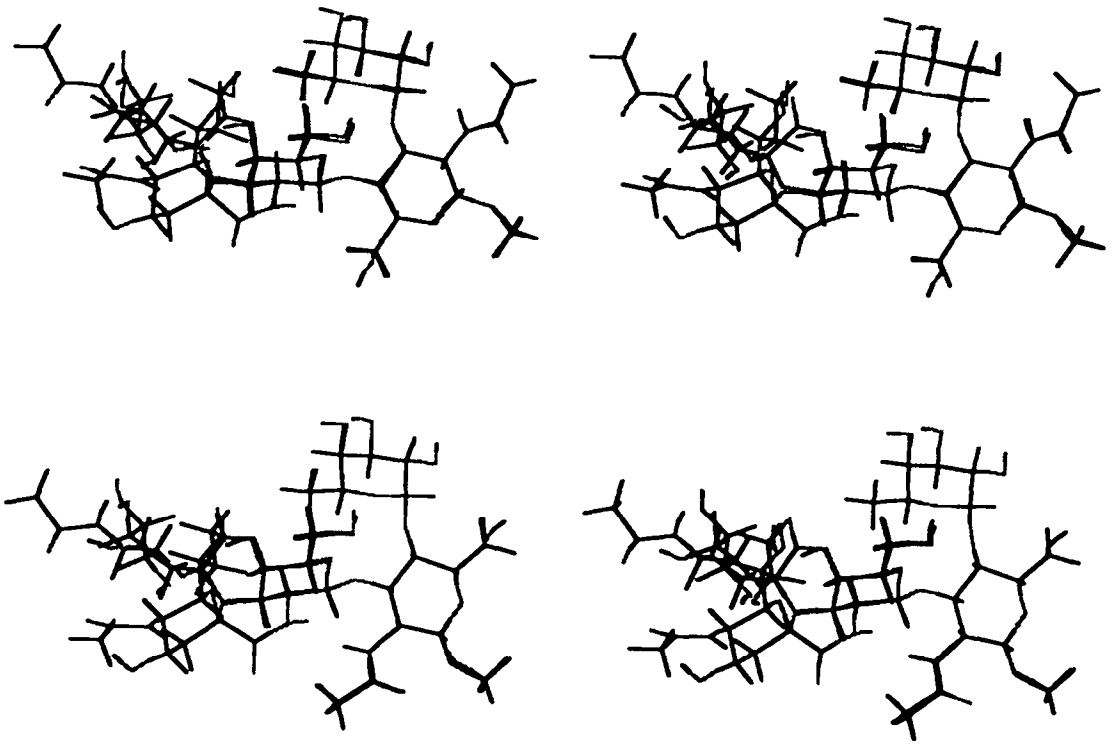

Figure 3. Stereoviews of the superimposed low-energy [MM2(91)] conformers of SLe^x-3,2- and 3,4-lactones (top) and SLe^a-3,2- and 3,4-lactones (bottom), obtained by RMS-fitting of the ring atoms of the Gal, Fuc, and GlcNAc residues.

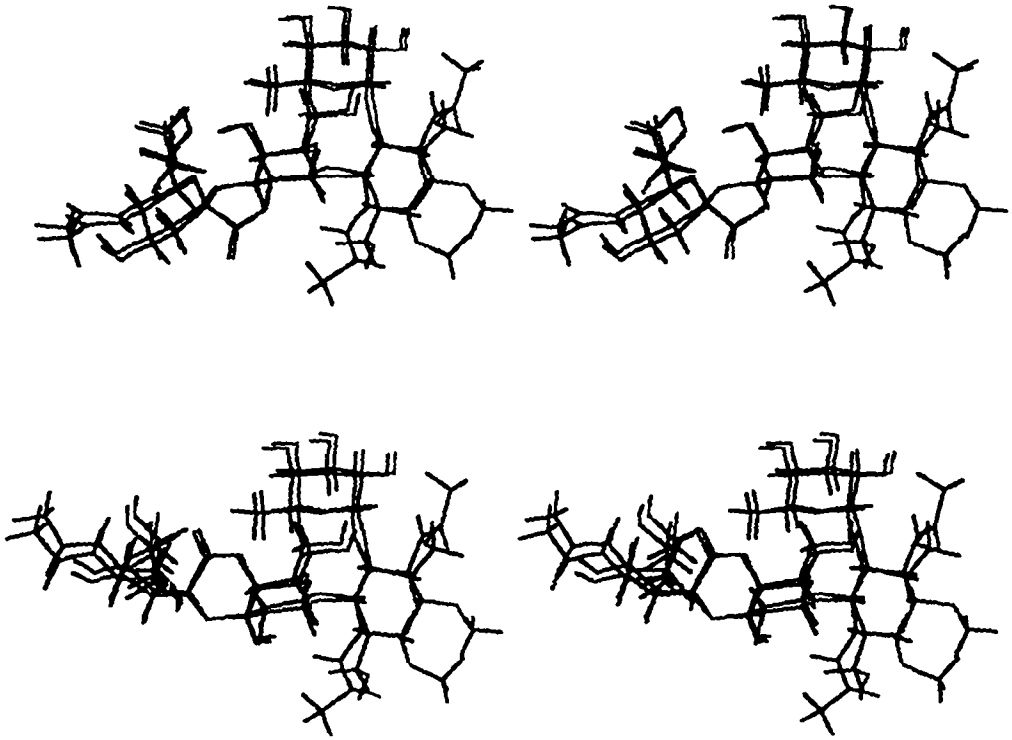

Figure 4. Stereoviews of the superimposed low-energy [MM2(91)] conformers of the SLe^x- and SLe^a-3,2-lactones (top) and the SLe^x- and SLe^a-3,4-lactones (bottom), obtained by RMS-fitting of the ring atoms of the NeuAc, lactone, Gal, Fuc, and GlcNAc residues.

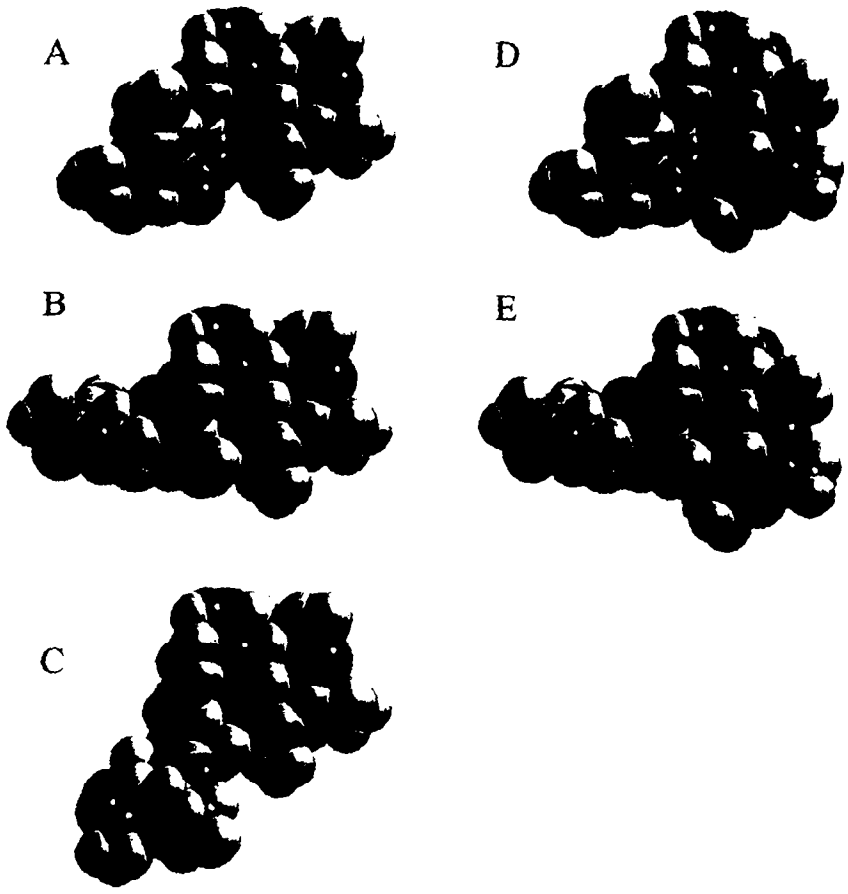

Figure 5. Space-filling models of A: SLe^x-3,2-lactone, B: SLe^x-3,4-lactone, C: SLe^x in the global energy minimum as defined in Table 2. D: SLe^a-3,2-lactone; E: SLe^a-3,4-lactone.

The dihedral driver option in the MM2(91) program[16] permitted a search of the NeuAc 'anomeric' conformational space of SLe^x. The Φ and Ψ angles were rotated independently with 30 ° increments and each conformation was automatically energy minimized with respect to all degrees of freedom except the dihedral angle used as the driver angle. Thus, the conformation corresponding to the global energy minimum obtained in the driver mode was finally minimized without any structural constraints. The resulting conformational map is shown in Figure 2.

The space-filling models (Fig. 5; standard atomic radii) were based on MacMimic coordinates (obtained from the energy minimizations).

Acknowledgment

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