PM3 and AM1 Study on β-N-acetyl-muramic Acid and 3 Murein Related Derivatives

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Abstract

The energetically favoured conformations of β-N-acetyl-Muramic acid, its C6-O-acetylated form, the methylamide and the methyl-glycoside have been investigated using the semiempirical PM3 and AM1 methods. All these compounds are either components or fragmentary structures of the murein network. The atomic coordinates of the starting set of the β-N-acetyl-Muramic acid molecule have been obtained by a PM3 minimization of one saccharide molecule cut out from the murein single strand model proposed by Barnickel at al. [1]. The sidegroups of the derivatives have been introduced by a molecular editor. These conformations served as starting points in conformational space for a grid search by scanning all sidechain torsional angles for non-hydrogen atoms with exception of the N-acetyl group which was held in cisoid position (i.e. N2-H bond is parallel to C1-H and C3-H bond) and only minimized. The PM3 method with an additional amide correction potential and the AM1 method were used. The torsional angle distributions of the lactyl sidechain (free acid and methylamide), the C6-O-acetylated sidechain and the C1-methoxy sidechain have been investigated, showing distinct energetically favoured torsional angle regions. The results are compared to earlier studies on β-N-acetyl-Muramic acid by J.S. Yadav et al. [2,3] who were using the MNDO and PCILO methods and by P.N.S. Yadav et al. [4] who were using the empirical MM2 force-field.

**Keywords:** Muramic Acid, Derivatives, Quantum Chemistry, Semiempirical Method, Conformational Energy

Introduction

β-N-acetyl-D-Muramic acid (NAM) and its C6-O-acetylated form are present in murein, a component of bacterial cell walls [5,6]. The C6-O-acetylated β-N-acetyl-D-Muramic acid (NAM-O6AC) has been found in the murein of Proteus mirabilis [7], Neisseria gonorrhoeae [8], Staphylococcus aureus [9] and 8 further species, see review [10]. It has been proposed, that O-acetylation is one factor for the reduced lysozyme degradability of murein [8,11]. The methylamide of NAM (NAM-MEAM) simulates the amide linkage between the saccharide and the peptide part in the murein network. Additionally the conformations of β-methylglycoside (NAM-O1ME) have been calculated.

The precise knowledge of the spatial structure of NAM and its derivatives is of interest for the molecular modelling of the murein network and especially for the drug design of muramyl peptide related compounds showing immuno-stimulating, somnogenic and pyrogenic activities [12]. The semiempirical methods AM1 and PM3 seem accurate enough to be applied to larger biological molecules [13,14,15] - larger

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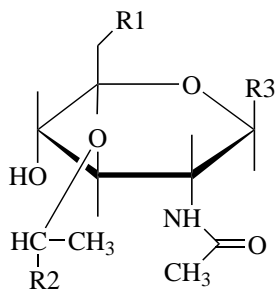
Figure 1. *Chemical formula of NAM and derivatives.*

in this quantum chemical context means equal or more than 10 non-hydrogen atoms. Their specific ability to find reasonable intramolecular hydrogen bonds makes these methods superior to the original MNDO parametrization.

Methods and Details of Calculations

Molecular modelling methods

In the present calculations the MNDO/PM3 method [16] with empirical correction potential for the torsional angle of the

amide bond and the AM1 method [17] are used. The calculations were performed on the IRIS/INDIGO (Silicon Graphics Inc.) cluster of our institute under the operating system IRIX System V.4 using SPARTAN, SGI versions 3.1.1 GL and 4.0.3 GL (Wavefunction, Inc.). Systematic grid search method was used for conformational analysis. From each grid point an energy minimization was started. Conformations with high energies were rejected.

Starting geometries and search parameters. The murein single strand model of Barnickel et al [1] has been used as starting set. The cartesian coordinates were kindly provided by the authors. One single NAM molecule in $^4C_1$ conformation has been cut out from the strand model, followed by a PM3 minimization. The starting conformations of the derivatives have been obtained by using the molecular editor implemented in SPARTAN to create the different side-chains. These conformations served as starting points in conformational space for a grid search by scanning all sidechain torsional angles for non-hydrogen atoms with exception of the N2-acetyl group which was minimized from cisoid position (i.e. N2-H bond is parallel to C1-H and C3-H bond). All amide bonds were set trans. Grid stepsize was set to 120° for all scanned torsional angles. The N2-acetyl group was not included in the angle scan but solely minimized, because X-ray structures of related compounds, like α-N-acetyl-Muramic acid [18], β-N,N'-diacetyl-Chitobiose trihydrate [19] and the co-crystallized complexes of muramyl-dipeptide (MDP) with isolectin I [20] and the β(1→4) connected trisaccharide NAM-NAG-NAM (NAG: N-acetyl-2-Glucos-amine) bound to hen egg white lysozyme [21], show that the favoured position of the N2-acetyl group is cisoid or, in the occurrence of hydrogen bonds, a slightly rotated position of the amide plane from cisoid position.
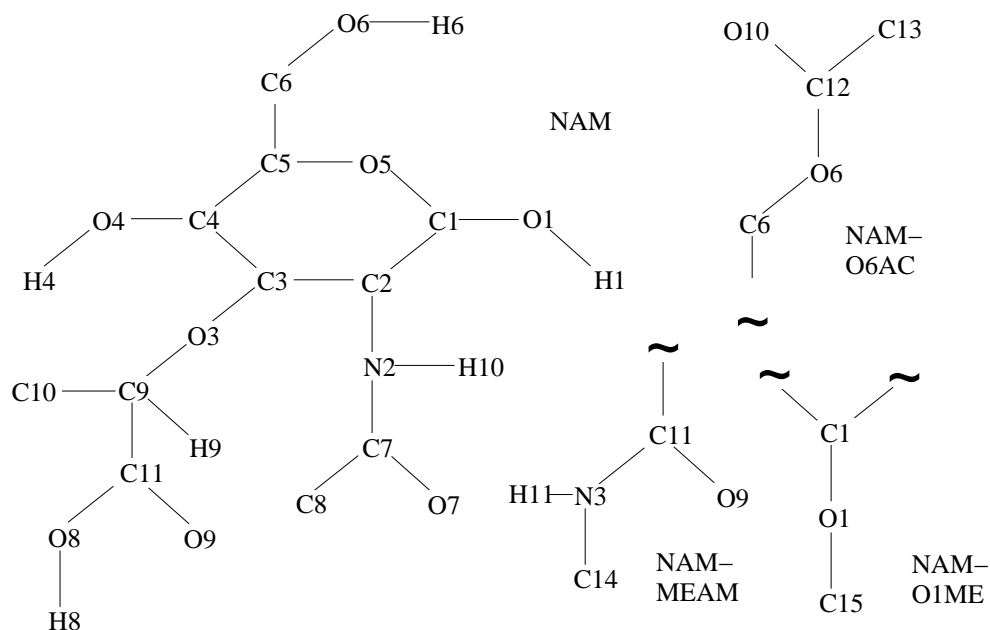

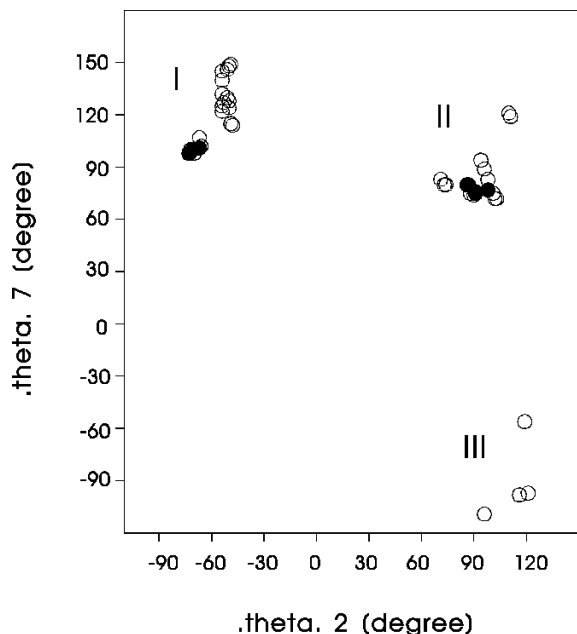**Figure 2.** *Numbering scheme for NAM and derivatives.*

Figure 3. *Scatterplot of the O3 (ether linkage) torsional angles (θ2 vs. θ7) of the 40 NAM-conformers with lowest energy calculated by the PM3 methode with amide correction potential (o) and the 10 NAM-conformers with lowest energy calculated by the AM1 methode (•). The roman numbers denote the energetically favoured torsional angle regions.*

Figure 4. *Scatterplot of the C9 (lactyl-.alpha.-C) torsional angles (θ7 vs. θ8) of the 40 NAM-conformers with lowest energy calculated by the PM3 methode with amide correction potential (o) and the 10 NAM-conformers with lowest energy calculated by the AM1 methode (•). Most of the PM3 conformers are equally distributed in a narrow angle band (dotted lines) θ7 ranging between 70° and 150°. The region III PM3-conformers of the ether linkage (O3) scatterplot are located in a separate area.*

Definitions

Figure 1 shows the chemical formula and figure 2 the numbering scheme for NAM and its derivatives. The definitions of the torsional angles are given in table 1. The torsional angles are defined by a sequence of 4 atoms. The second bond between atom 2 and 3 is the axis of rotation. A positive rotation is then defined as the rotation of the third bond between atom 3 and 4 in a counter-clockwise direction viewed from the moving bond (between atom 3 and 4) towards the fixed bond (between atom 1 and 2). (SPARTAN implemented signum definition)

Results

β-N-acetyl-D-Muramic acid

Listing of low-energy conformers of NAM. Table 2 lists the 10 lowest energy conformers calculated by the PM3 method with amide correction potential and the AM1 method. Additionally, the reference data of J.S. Yadav et al. [2,3] and P.N.S. Yadav et al. [4] are given in table 2. The conformers are numbered with rising energy. To distinguish between PM3 and AM1 conformers, the AM1 conformers are marked with an
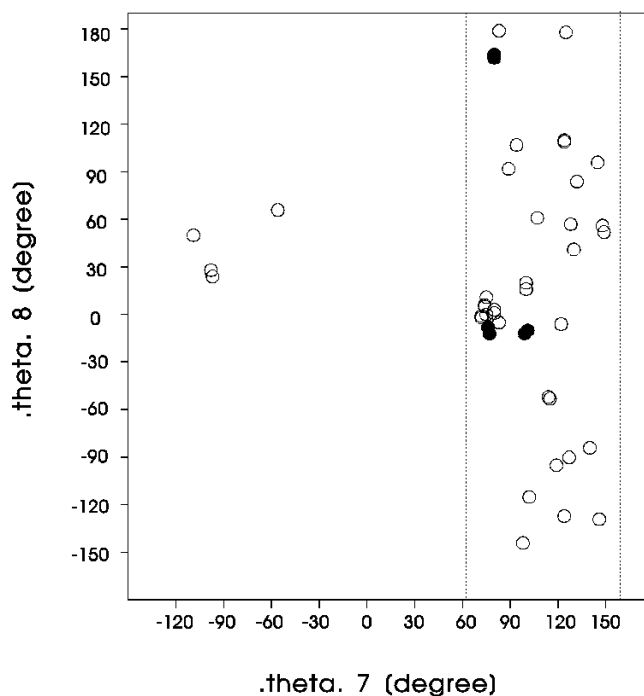
apostrophe. The absolute energy difference between the lowest energy conformer of PM3 and AM1 is considerable and amounts to 25 kcal/mol.

Calculated hydrogen bonding patterns. The hydrogen bonds of the first five conformers calculated by both methods are shown in table 3. Normally PM3 gives smaller values for the H-bond distance, ranging between 1.80 Å and 1.83 Å, compared to the AM1 values which range from 2.14 to 2.21 Å. The carboxylic acid group can form stable hydrogen bonds either to the C4-hydroxyl group or to the N2-H of the amide group, leading to a restriction of the conformational flexibility of the lactyl group. In case of hydrogen bonding, the amide group deviates slightly from planarity in some AM1 conformers. PM3 conformers are uneffected because of the usage of the additional empirical amide torsional angle potential.

Torsional angle distribution at the O3 ether linkage. According to our calculations the sterical requirements of the O3 ether linkage between pyranosyl ring and D-lactyl group are quite restrictive. Figure 3 shows the scatterplot (θ2 vs. θ7) of the O3 torsional angles. The 40 lowest PM3 energy

Table 1. *Definition of torsional angles*

No.	NAM	NAM-O6AC	NAM-MEAM	NAM-O1ME
1	C1-C2-N2-C7			
2	C4-C3-O3-C9			
3	C5-C4-O4-H4			
4	O5-C5-C6-O6			
5	C5-C6-O6-H6	C5-C6-O6-C12	C5-C6-O6-H6	C5-C6-O6-H6
6	O5-C1-O1-H1	O5-C1-O1-H1	O5-C1-O1-H1	O5-C1-O1-C15
7	C3-O3-C9-C11			
8	O3-C9-C11-O9			
9	C9-C11-O8-H8	C9-C11-O8-H8	C9-C11-N3-C14	C9-C11-O8-H8
10	–	C6-O6-C12-O12	–	–

NAM: β-N-acetyl-Muramic acid
NAM-O6AC:C6-O-acetylated β-N-acetyl-Muramic acid
NAM-MEAM: Methylamide of β-N-acetyl-Muramic acid
NAM-O1ME: Methylglycoside of β-N-acetyl-Muramic acid

Table 2. *The torsional angles and absolute and relative energies of the calculated first 10 low-energy conformers of NAM by PM3 and AM1 methode are listed. θ 9 allways minimized to the value of 180°. Therefore it is not included in the table.*

Conf. No.	θ [°] 1	2	3	4	5	6	7	8	Energy [kcal/mol]	ΔE [kcal/mol]
PM3:										
1	109	-49	-168	72	-62	70	115	-53	-353.60	0.0
2	69	101	-49	79	-63	126	75	0	-353.28	0.32
3	114	-71	-176	72	-63	68	100	20	-352.91	0.69
4	105	-50	-169	72	-62	72	124	109	-352.89	0.71
5	109	116	-173	72	-62	69	-98	28	-352.64	0.96
6	98	-54	-175	73	-62	76	145	96	-352.52	1.08
7	93	74	-48	80	-63	83	80	3	-352.49	1.11
8	72	88	49	75	-64	119	75	11	-352.45	1.15
9	84	103	-50	79	-63	85	72	-2	-352.42	1.18
10	115	-70	178	73	-63	68	98	-144	-352.17	1.43
AM1:										
1'	87	91	-63	171	-69	76	76	-8	-379.49	0.0
2'	87	91	-74	-67	61	77	75	-8	-379.33	0.16
3'	87	91	-63	65	-57	76	75	-8	-378.92	0.56
4'	87	86	-63	171	-69	75	80	164	-378.37	1.12
5'	87	87	-74	-68	62	76	80	163	-378.19	1.30
6'	87	87	-64	65	-57	75	80	162	-377.76	1.73
7'	87	98	-161	157	57	74	77	-12	-377.43	2.06
8'	80	-72	-174	66	-58	67	99	-12	-376.39	3.10
9'	80	-67	-170	-55	61	69	101	-10	-376.32	3.17
10'	80	-73	-174	157	56	65	98	-12	-376.24	3.24

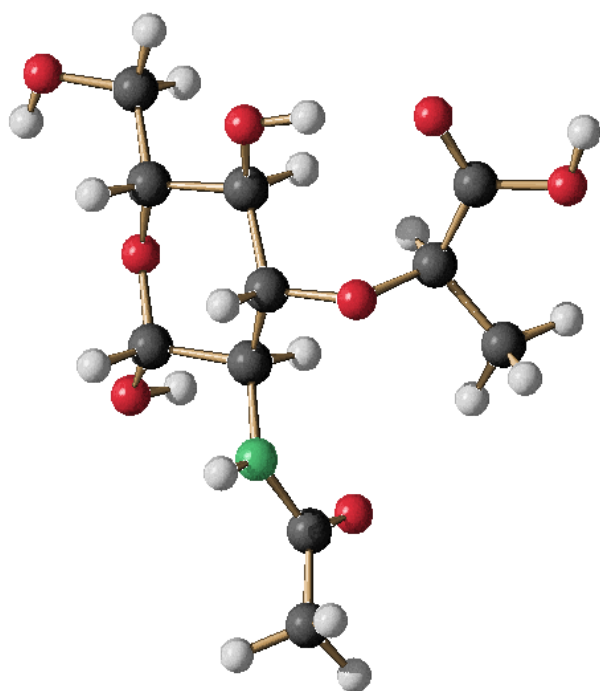| (ref.data) Methode	1	2	3	4	5	6	7	8	Energy	Reference
MNDO	113	99	-97	159	-165	-61	98	53	Minimum	[2]
PCILO	173	142	-54	-168	-177	-61	139	-142	Minimum	[3]
MM2	100	-86	56	-179	162	104	-78	-84	Minimum	[4]

Figure 5. *Molecular graphic of NAM-conformer 1, the energy minimum conformer calculated with the PM3 methode with amide correction potential. This and all following molecular graphics have been created using the program SCHAKAL88 (E. Keller, Universitaet Freiburg, Germany, 1988).*
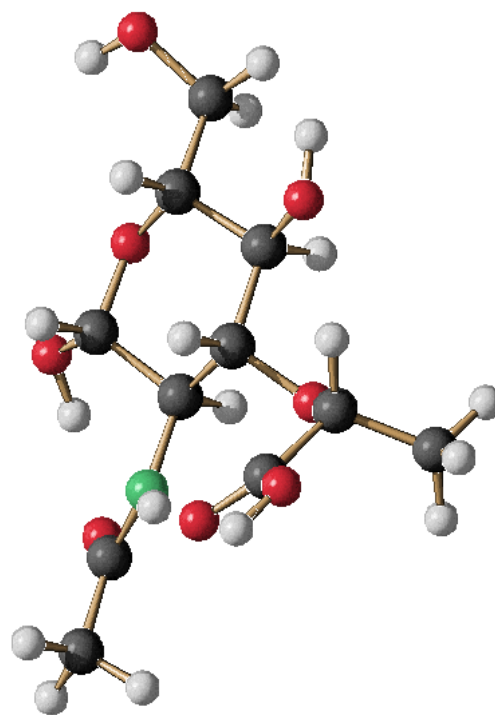

Figure 6. *Molecular graphic of NAM-conformer 2, the second lowest energy PM3 conformer. The N-acetyl group forms two hydrogen bonds to neighbouring side chains.*

conformers up to a relative energy of 2.4 kcal/mol and the 10 lowest AM1 energy conformers up to a relative energy of 3.2 kcal/mol are shown in the distribution. PM3 and AM1 low energy conformers are found in the torsional angle regions I and II (see figure 3). Considering the corresponding energies, the region I is slightly favoured by PM3, whereas region II is strongly favoured by AM1. A small number of PM3 conformers with relative energies of more than about 1 kcal/mol over minimum are accumulated in a third more diffuse region III (figure 3). No AM1 conformers with reasonable energy can be found there. Ether linkage torsional angle regions (PM3): I : $\theta2= -60° +/- 15°$, $\theta7= 120° +/- 30°$ II : $\theta2= 90° +/- 20°$, $\theta7= 90° +/- 30°$ III: $\theta2= 105° +/- 15°$, $\theta7= -80° +/- 30°$.

Torsional angle distribution at lactyl α-carbon. Figure 4 shows the torsional angle distribution at C9, the α-C-atom of the lactyl sidechain, as scatterplot. The same conformers as in the previous plot have been taken into account. All PM3 conformers with exception of the earlier mentioned region III conformers are equally distributed in a small angle band, $\theta7$ ranging from 70° to 150°. The carboxylic acid plane may assume any rotational angle ($\theta8$), as long as $\theta7$ is inside the allowed angle band, according to the PM3 results. In the AM1 calculation, we found, that the carboxylic acid plane rota-

tional angle is fixed, allowing only the ~170° and the ~-10° positions. $\theta7$ is restricted to two values, one about 80°, the other around 100°. Thus, the AM1 results indicated a quite rigid geometry of the α C torsional angles.

NAM conformer 1 (figure 5): The calculated PM3 energy of the energy minimum conformer is -353.60 kcal/mol. In the ether linkage torsional angle scattering plot this conformer is inside region I. One hydrogen bond is situated between the hydroxyl group at C4 and the carbonyl oxygen of the acid (O9). The N-acetyl group is positioned strictly cisoid. The C6-O6 bond of the hydroxymethyl group is gauche to C5-O5 bond and trans to C5-C4 bond.

NAM conformer 2 (figure 6): A PM3 energy of -353.28 kcal/mol has been calculated. Conformer 2 is the lowest PM3 energy conformer of region II (ether linkage). The N-acetyl group forms two hydrogen bonds: One between N2-H10 and the carbonyl oxygen of the acid (O9), the other between the carbonyl oxygen of the amide (O7) and the anomeric hydroxyl group. These two hydrogen bonds force the amide plane of the N-acetyl group out of cisoid position, slightly rotated in a clockwise direction (viewed from the moved side-group towards the fixed pyranose ring) by 50°. Thus positioning the N2-C7 bond nearly trans to C2-C3 bond. Like in con-

Table 3. *PM3 calculated H-bonds are generally shorter than AM1 H-bonds and the angles are greater compared to the more acute angles calculated by AM1, PM3 conformer 3 being an exception which may not meet the strict definitions of an hydrogen bond.*

Conf. No.	Atoms	Angle [°]	Distance [Å]
PM3:			
1	O4-H4...O9	168	1.81
2	O1-H1...O7	151	1.80
	N2-H10...O9	164	1.83
3	O4-H4...O9	144	2.48
4	O4-H4...O8	167	1.83
5	O4-H4...O9	161	1.80
AM1:			
1'	O4-H4...O6	128	2.15
	N2-H10...O9	154	2.18
2'	N2-H10...O9	154	2.18
3'	N2-H10...O9	153	2.18
4'	O4-H4...O6	128	2.14
	N2-H10...O8	150	2.21
5'	N2-H10...O8	150	2.21

former 1, the C6-O6 bond is gauche to C5-O5 bond and trans to C5-C4 bond.

NAM conformer 1' (figure 7): The calculated AM1 energy is -379.49 kcal/mol. 25.89 kcal/mol less than the PM3 energy minimum conformer. The lowest AM1 energy conformer is found in region II of the ether linkage plot. The molecule shows two intramolecular hydrogen bonds. One between N2-H10 of the amide and the carbonyl oxygen of the acid (O9). The second between O6 and the hydroxyl group at C4. The amide plane of the N-acetyl group is rotated out of cisoid position in clockwise direction by 48°. The C6-O6 bond is positioned trans to C5-O5 bond and gauche to C5-C4 bond.

C6-O-acetylated β-N-acetyl-D-Muramic acid

Listing of low-energy conformers of NAM-O6AC. The 10 energetically most favoured conformers of NAM-O6AC calculated by PM3 method with an additional empirical amide potential and AM1 method (conformer numbers marked with an apostrophe) are listed in table 4. Torsional angles θ5 and θ10 define the newly introduced ester group at C6 (definitions see figure 1 and table 1).

Calculated hydrogen bonds. Table 5 lists the hydrogen bonding parameters of the 5 lowest energy conformers of NAM-O6AC calculated by both methods. According to the PM3 results, the ester group is not involved in hydrogen bond formation. The hydrogen bond between O4-H4 and the

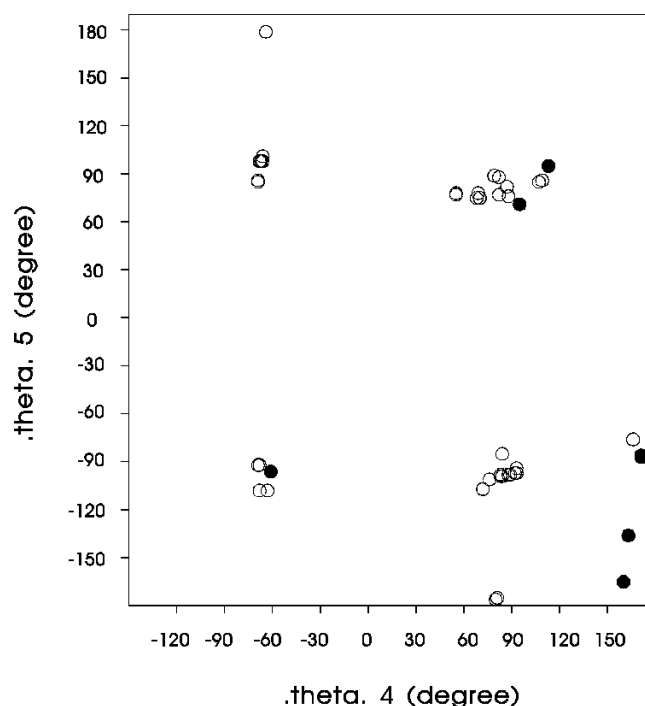**Figure 7.** *Molecular graphic of NAM-conformer 1', the energy minimum conformer calculated by AM1 methode.*

Figure 8. *Scatterplot of C6 torsional angles (ester group, θ4 vs. θ 5) of NAM-O6AC. Definition of symbols see legends of figures 3 and 4.*

Table 4. *The torsional angles and absolute and relative energies of the calculated first 10 low-energy conformers of NAM-O6AC by PM3 and AM1 methode are listed. As in table 2, the values for θ 9 are omitted, because they allways minimized to 180°.*

Conf. No.	1	2	3	4	θ [°] 5	6	7	8	10	Energy [kcal/mol]	ΔE [kcal/mol]
PM3:											
1	123	-67	-167	68	75	12	98	-7	177	-393.31	0.0
2	114	-67	-167	83	-99	71	99	-10	178	-393.15	0.16
3	123	-67	-167	79	89	11	98	-7	-179	-393.11	0.20
4	107	-48	-165	-67	98	74	115	-51	-175	-393.09	0.22
5	112	-65	-164	-66	98	72	99	-8	-176	-393.04	0.27
6	123	-67	-167	76	-101	15	98	-7	-5	-392.88	0.43
7	108	-50	-168	83	-98	73	116	-54	178	-392.87	0.44
8	123	-67	-168	55	78	15	98	-7	-1	-392.78	0.53
9	110	-49	-168	70	75	65	115	-53	176	-392.73	0.58
10	122	-67	-166	80	-176	15	98	-7	0	-392.70	0.61
AM1:											
1'	87	91	28	95	71	77	75	-8	-4	-416.91	0.0
2'	87	91	-61	113	95	76	76	-8	-1	-416.88	0.03
3'	87	92	-80	-61	-96	75	76	-8	3	-415.99	0.91
4'	87	86	29	95	71	76	80	164	-4	-415.85	1.05
5'	87	86	-60	113	95	74	80	163	-1	-415.75	1.16
6'	87	92	-61	171	-87	75	75	-8	2	-415.69	1.22
7'	87	91	-51	160	-165	75	75	-8	-2	-415.36	1.55
8'	87	91	-54	163	-136	75	75	-8	-1	-415.31	1.60
9'	87	87	-81	-61	-96	74	81	162	4	-414.90	2.01
10'	87	87	-61	171	-86	74	80	163	2	-414.62	2.29

carbonyl-oxygen of the lactyl group (O9) is similar to the one found in the not O6-acetylated NAM. Whereas the AM1 method favours one hydrogen bonding pattern involving the ester carbonyl-oxygen (O10) of the O-acetyl group. As in the NAM case, the H-bond distances calculated by the PM3 method are considerably shorter (by 0.3 A) than the respective AM1 values.

Differences in lactyl sidechain conformations of NAM-O6AC in comparison to NAM. Ether linkage torsional angles (θ2 and θ7) for NAM-O6AC calculated by the PM3 method are found predominantly in region I. In contrast, the AM1 results are found nearly exclusively in region II. A similar difference, although less pronounced, was observed for the NAM results. Region III conformers have not been found for reasonable energies by neither method. The torsional angle distribution at the lactyl-α-carbon (C9;θ7,θ8) is very similar to the NAM results, but again no region III conformers could be observed.

Torsional angle distribution at C6-O-acetyl sidechain. C6 torsional angle values are widely distributed according to the calculations by both methods , no preference could be detected (figure 8,θ4 vs. θ5). Both methods favour a planar ester group geometry. AM1 results give the cis orientation (Def.: C-O-C=O) for the ester torsional angle θ10, thus allowing an intramolecular hydrogen bond between the ester carbonyl-oxygen (O10) and the neighboured hydroxylgroup at C4. PM3 method slightly favours the trans geometry (θ10) for the ester group by about 0.5 kcal/mol.

AM1 and PM3 energy minimum conformers of NAM-O6AC in detail. The lowest energy conformers calculated by both methods are presented below.

NAM-O6AC conformer 1 (figure 9): The calculated PM3 energy using additional amide potential is -393.31 kcal/mol. The ether linkage belongs to region I. A hydrogen bond between O4-H4 and the carbonyl-oxygen of the lactyl group

Table 5. *PM3 and AM1 calculated hydrogen bonds of NAM-O6AC are listed. In correspondence to the NAM case, the PM3 calculated hydrogen bonds are shorter by 0.3 to 0.4 Å. than the AM1 values.*

Conf. No.	Atoms	Angle [°]	Distance [Å]
PM3:			
1	O4-H4...O9	156	1.81
2	O4-H4...O9	157	1.82
3	O4-H4...O9	156	1.81
4	O4-H4...O9	168	1.81
5	O4-H4...O9	158	1.81
AM1:			
1'	O4-H4...O10	142	2.22
	N2-H10...O9	153	2.20
2'	O4-H4...O10	149	2.13
	N2-H10...O9	154	2.18
3'	O4-H4...O10	140	2.18
	N2-H10...O9	153	2.16
4'	O4-H4...O10	141	2.22
	N2-H10...O8	150	2.23
5'	O4-H4...O10	149	2.13
	N2-H10...O8	150	2.21

Figure 9. *Molecular graphic of NAM-O6AC conformer 1, the lowest energy conformer by PM3 methode with amide correction potential.*

(O9) is formed. The amide group is strictly cisoid. The ester bond is trans referred to θ10 (C-O-C=O).

NAM-O6AC conformer 1' (figure 10): The calculated AM1 energy is -416.91 kcal/ mol. The ether linkage torsional angles belong to region II. Two hydrogen bonds are formed, one between the N2-H10 of the amide group and the carbonyl-oxygen of the lactyl group (O9), the other between the hydroxyl group at C4 (O4-H4) and the carbonyl-oxygen of the ester group (O10). The amide plane of N-acetyl group is rotated out of cisoid position in a clockwise direction by 50° (viewed from the moved side-group towards the fixed pyranose ring).

Methylamide of β-N-acetyl-D-Muramic acid

Listing of low-energy conformers of NAM-MEAM. Table 6 lists the low-energy conformers of NAM-MEAM calculated by both semiempirical methods. The absolute energy difference between the energy minimum conformers calculated by PM3 and AM1 is 22 kcal/mol.

Calculated hydrogen bonding patterns and amide-amide π-complex structures. The energy minimum conformer calculated by PM3 with additional amide correction potential (conformer 1) shows an interesting chained hydrogen bonding structure (figure 11 and table 7). The C6 hydroxyl group
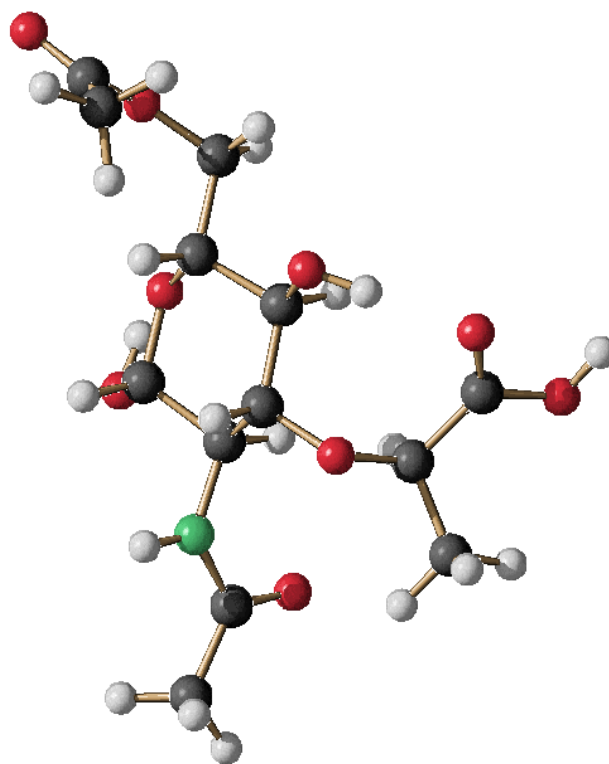
is directed towards the O4 and the C4 hydroxylgroup is pointing towards the carbonyl oxygen of the amidated lactyl group (O9). This structure is energetically in concurrence to the formation of an amide-amide π-complex found in PM3-conformer 3 with a relative energy rise of only 0.16 kcal/mol. However it must be stated, that the molecules are calculated in vacuo. Thus, such a complex would probably not be stable if intermolecular hydrogen bonds can be formed. The precise geometry of the amide-amide π-complex is given in figure 12. The AM1 energy minimum conformers of NAM-MEAM and NAM form the same stable O4-H4...O6 H-bond. Additionally an intramolecular interaction between both amide groups via a hydrogen bond is calculated for the AM1 minimum conformer of NAM-MEAM.

NAM-MEAM conformations of the amidated lactyl group at C3. The ether linkage torsional angle distribution shows similar features as the free acid form (NAM). However, according to the AM1 results, most energetically reasonable conformers are accumulated in the region II of the O3 torsional angle distribution plot. No AM1 conformers could be found in the region III, as in the NAM case. PM3 results indicate a split of region II into two subregions, subregion IIA (θ2= 100° +/-10°, θ7= 90° +/-10°) and subregion IIB

Table 6. *The torsional angles and absolute and relative energies of the calculated first 10 low-energy conformers of NAM-MEAM by PM3 and AM1 methode are listed. θ9 allways minimized to the value of 180° (PM3) rsp. 180° +/- 4° (AM1) and is not included in the table.*

Conf. No.	θ [°]								Energy [kcal/mol]	ΔE [kcal/mol]
	1	2	3	4	5	6	7	8		
PM3:										
1	119	-67	-170	173	42	65	97	-1	-303.07	0.0
2	109	-50	-171	72	-61	70	117	-48	-302.96	0.11
3	111	113	179	73	-63	71	118	-128	-302.91	0.16
4	117	-67	-169	72	-62	67	97	0	-302.82	0.25
5	106	-50	-161	72	-63	72	123	-114	-302.20	0.87
6	111	-47	-162	-66	-49	64	117	-74	-301.93	1.14
7	124	-68	-169	-65	-49	15	95	1	-301.90	1.17
8	111	-50	-171	-67	-49	65	116	-47	-301.64	1.43
9	116	113	178	-65	-45	65	118	-129	-301.33	1.74
10	109	116	-177	72	-62	69	-102	38	-301.18	1.89
AM1:										
1'	87	89	-62	170	-68	77	76	-8	-325.06	0.0
2'	82	148	179	66	-59	73	107	-126	-325.04	0.02
3'	123	134	177	68	-60	48	153	149	-323.86	1.20
4'	124	135	177	157	60	45	154	148	-323.70	1.36
5'	85	106	-173	66	-58	74	84	-66	-323.60	1.46
6'	87	97	-164	66	-57	76	83	-34	-323.58	1.48
7'	84	141	-71	175	-67	74	111	-120	-323.45	1.61
8'	82	148	-170	-143	-56	71	106	-127	-323.42	1.64
9'	85	93	-65	173	-68	72	86	-139	-322.91	2.15
10'	82	-67	60	71	-62	71	91	-153	-322.84	2.22

(θ2= 140° +/-10°, θ7= 100-150°), plots not shown. Both methods gave very similar results concerning the torsional angle distribution at the lactyl-α-carbon C9 (θ7 vs. θ8). Most of the energetically reasonable conformers are enclosed in a rectangle θ7= 90 - 130°, θ8= -140 - 0° (PM3) rsp. θ7= 75 - 115°, θ8= -150 - 0° (AM1).

NAM-MEAM conformer 1 (figure 11): The calculated PM3 energy of NAM-MEAM conformer 1 amounts to -303.07 kcal/mol. The N2-acetyl group is cisoid and not involved in hydrogen bonding. The amide group of the lactyl sidechain is bonded to a chained hydrogen bond between the hydroxylgroup at C6, the hydroxylgroup at C4 and the carbonyl oxygen of the amide (O6-H6...O4-H4... O9). The ether torsional angles belong to region I. C6-O6 bond is trans to C5-O5 and gauche to C5-C4.

NAM-MEAM conformer 1' (figure 13): NAM-MEAM conformer 1' has an AM1-energy of -325.06 kcal/mol. The N2-acetyl group is rotated out of cisoid position by 50° (in clockwise direction when viewed from the moving side-group towards the fixed pyranose ring), forming a hydrogen bond to the carbonyl oxygen of the lactyl-amide group (N2-H10...O9). The ether torsional angles belong to region II. C6-O6 bond is trans to C5-O5 and gauche to C5-C4.

Methylglycoside of β-N-acetyl-D-Muramic acid

Listing of low-energy conformers of NAM-O1ME. Table 8 lists the calculated low-energy conformers of NAM-O1ME. θ6 is the glycosidic torsional angle of the methylglycoside. PM3 favours a value of 70° for θ6. Values of -98° +/- 2° are found in conformer 2,8 and 10. AM1 strongly favours the values of 64° +/- 2° for θ6. The N2-acetyl group is positioned cisoid by both methods. O3 sidechain conformations are very similar to NAM.

Table 7. *PM3 and AM1 calculated hydrogen bond geometries of NAM-MEAM are listed. Some conformers do not form hydrogen bonded structures, instead conformer 3 (PM3) shows the formation of an amide-amide pi-complex.*

Conf. No.	Atoms	Angle [°]	Distance [Å]
PM3:			
1	O6-H6...O4	143	1.85
	O4-H4...O9	157	1.80
2	O4-H4...O9	167	1.79
3	-		
4	O4-H4...O9	157	1.81
5	-		
AM1:			
1'	O4-H4...O6	128	2.15
	N2-H10...O9	152	2.15
2'	-		
3'	N3-H11...O7	141	2.23
4'	O6-H6...O4	129	2.22
	N3-H11...O7	142	2.22
5'	N3-H11...O7	146	2.17

Figure 10. *Molecular graphic of NAM-O6AC conformer 1', the lowest energy conformer by AM1 methode.*

Hydrogen bonding patterns. Hydrogen bonding is less variable. PM3 calculates a hydrogen bond between O4-H4 and the carbonyl oxygen of the lactyl group (O9) in the 4 lowest-energy conformers. AM1 found the hydrogen bond between N2-H10 (amide) and the carbonyl oxygen of the lactyl group in the conformers 1',2',3' and 5'.

NAM-O1ME conformer 1 (figure 14): Calculated PM3 energy = -345.91 kcal/mol. N2-acetyl group is cisoid. Ether linkage belongs to region I. C6-O6 bond is gauche to C5-O5 bond and trans to C5-C4 bond. O1-C15 bond (C15 = aglycan) is situated parallel to C2-H bond. Torsional angle values are very close to NAM PM3-conformer 1.

NAM-O1ME conformer 1' (figure 15): The AM1 energy amounts to -371.34 kcal/mol. Torsional angle values are very close to the corresponding NAM AM1-conformer 1'.

The introduction of the methyl group at O1 had only little effect on the overall structures of the energy minimum conformers calculated by both methods.

Discussion

Semiempirical quantum chemical methods have been successfully applied to monosaccharides and monosaccharide related compounds, e.g. β-D-fructo-pyranose using AM1 and MNDO/M [14], 2-methoxytetrahydropyran using PM3 and
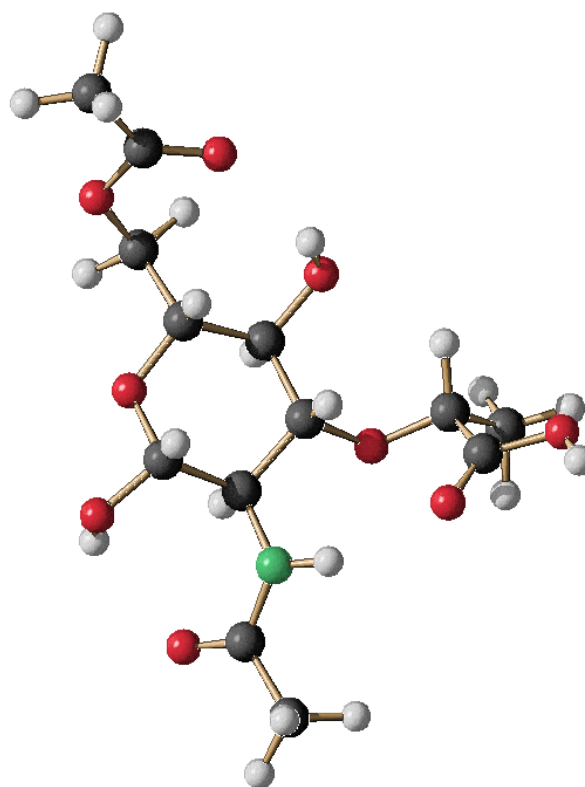
AM1 [15]. In the case of β-D-fructopyranose, the AM1 procedure performed better on the intramolecular interactions than the MNDO/M procedure. In respect to the puckering parameters for the ring shape and the average torsional angle around the pyranose ring, the AM1 values were in close agreement with the values of the crystal conformation in contrast to the MNDO/M values. The calculations on β-D-fructopyranose suggest that AM1 handles O-H...O hydrogen bonding better than MNDO/M [14]. Scano and Thomson [15] calculated the heats of formation for 19 large molecules with PM3 and AM1 methods, to test the application of these methods to larger biological molecules. The monosaccharide related compound 2-methoxytetrahydropyran was calculated and compared to experimental results. PM3 gave results of higher accuracy. So it seemed reasonable to apply AM1 and PM3 methods for the calculation of the conformational structure of NAM and its derivatives (NAM-O6AC, NAM-MEAM, NAM-O1ME).

β-N-acetyl-Muramic acid. In this study, distinct hydrogen bonding patterns have been calculated for the NAM molecule in β-form (table 3). Knox and Murthy [18] reported in their crystallographic study on the α-form an intramolecular hydrogen bond between the N-H bond of the amide and

Table 8. *The torsional angles and absolute and relative energies of the calculated first 10 low-energy conformers of NAM-O1ME by PM3 and AM1 methode are listed.*

Conf.	θ [°]								Energy	ΔE
No.	1	2	3	4	5	6	7	8	[kcal/mol]	[kcal/mol]
PM3:										
1	113	-49	-168	72	-62	70	115	-53	-345.91	0.0
2	114	-49	-167	70	-65	-98	115	-54	-345.16	0.75
3	118	117	-175	72	-62	69	-97	27	-344.99	0.92
4	112	-50	-171	-75	-70	69	115	-53	-344.33	1.58
5	115	112	-177	72	-63	70	120	-104	-344.30	1.61
6	109	-53	-173	72	-63	70	131	82	-344.27	1.64
7	117	96	-160	72	-62	70	-109	49	-344.26	1.65
8	108	-54	-175	71	-65	-100	144	96	-344.00	1.91
9	112	108	-175	72	-63	70	120	87	-343.89	2.02
10	124	118	-175	70	-65	-96	-97	27	-343.84	2.07
AM1:										
1	116	92	-65	172	-67	64	74	-11	-371.34	0.0
2	116	99	-162	67	-58	65	75	-16	-370.23	1.11
3	120	100	-166	65	-58	66	84	-23	-369.99	1.35
4	116	-78	-173	67	-59	62	92	-5	-369.43	1.91
5	118	93	-77	-69	59	-74	73	-12	-368.97	2.37
6	119	92	-67	173	-65	-73	73	-12	-368.83	2.51
7	125	-75	-174	63	-61	-67	96	-9	-368.25	3.09
8	115	-64	-174	-133	-57	62	106	-15	-368.13	3.21
9	117	-76	-176	-117	-61	61	92	1	-368.10	3.24
10	122	99	-163	64	-60	-72	74	-17	-368.00	3.34

carbonyl oxygen of the lactyl group. The PM3 and AM1 calculations, presented here, also suggest such an hydrogen bond for the β-anomer. The lactyl sidechain backbone geometry is determined by the three torsional angles θ2, θ7 and θ8. These angles are quite restricted to certain values (AM1) or angle ranges (PM3). AM1 strongly favours the angle triplet (θ2= 90° +/- 4°, θ7= 80° +/- 5°, θ8= -10° or +165° +/- 2°) up to a relative energy of 2 kcal/mol, whereas PM3 allows certain angle regions (see figs. 3,4).

The N2-acetyl sidechain torsional angle θ1 is minimized to the value of 87° according to the AM1 results, for all low energy conformers. PM3 shows a greater variation between strict cisoid position (θ1 = 109°), if no hydrogen bonds are formed, up to a value of θ1 = 69°, in case of two hydrogen bonds of the amide group to neighbouring side groups.

Pincus et al. [22] found the anti-cisoid conformation of the N-acetyl group (θ1= -60°) with the amide carbonyl oxygen coming into close proximity to the hydrogens at C1 and C3 to be energetically favoured for the NAM molecule. For better comparison of our results to the results of empirical conformational energy calculations by Pincus et al. [22], we

used the energy minimum conformer of the authors, for a single point and an energy minimization with PM3 and AM1 methods. We found, that the energy minimum conformer for the NAM molecule proposed by Pincus et al. [22] has a relative energy of more than 10 kcal/mol (PM3) and nearly 20 kcal/mol (AM1). Even after optimization of the Pincus model the energy values were still 5 kcal/mol above our calculated minimum structures. Thus, the anti-cisoid conformation of the N-acetyl group will not be discussed further in this study.

J.S. Yadav et al. [2,3] investigated the conformational structure of NAM using the semiempirical quantum chemical methods MNDO and PCILO. In the lowest MNDO energy conformer of NAM (see table 2, ref. data) the N-acetyl group is in cisoid position (θ1= 113°) in agreement to our PM3 results. However, MNDO was unable to find hydrogen bonds. Thus, the lactic acid group is not involved in hydrogen-bond formation with its neighbouring side chains, as it is the case with the N-acetyl group [2]. In contrast, the PCILO calculations on NAM showed the formation of hydrogen bonds between C4-hydroxyl group and carboxylic acid (O4-H4...O9 and O4-H4...O6) [3]. In the present study various
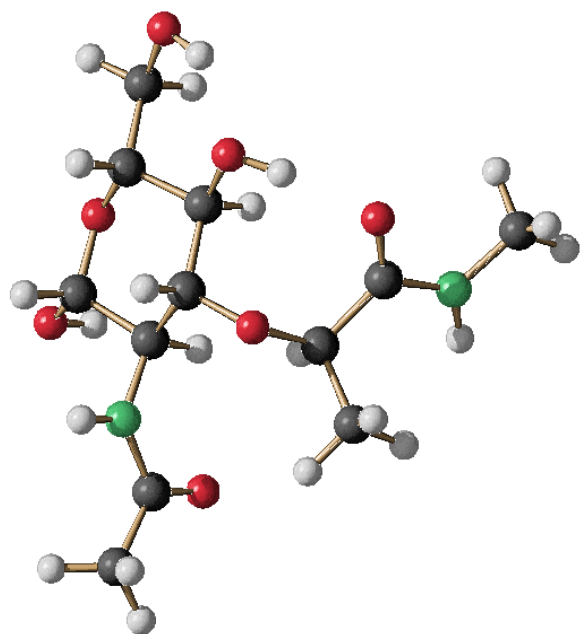
Figure 11. *Molecular graphic of NAM-MEAM conformer 1, the energy minimum conformer calculated by PM3 methode with amide correction potential.*

Table 9. *PM3 and AM1 calculated hydrogen bond geometries of NAM-O1ME are listed.*

Conf. No.	Atoms	Angle [°]	Distance [Å]
PM3:			
1	O4-H4...O9	168	1.81
2	O4-H4...O9	168	1.81
3	O4-H4...O9	161	1.79
4	O4-H4...O9	168	1.80
5		–	
AM1:			
1	N2-H10...O9	138	2.24
2	N2-H10...O9	136	2.20
3	N2-H10...O9	133	2.20
4	O4-H4...O9	133	2.13
5	N2-H10...O9	137	2.25

hydrogen bonding patterns have been found (see table 3), including those proposed by J.S. Yadav et al. [3]. The MM2 calculations of P.N.S. Yadav et al. [4] are only of limited value, because, despite the low computation time needed for such calculations, they did no grid search, using only the conformers calculated by Pincus et al. [22] as starting geometries.
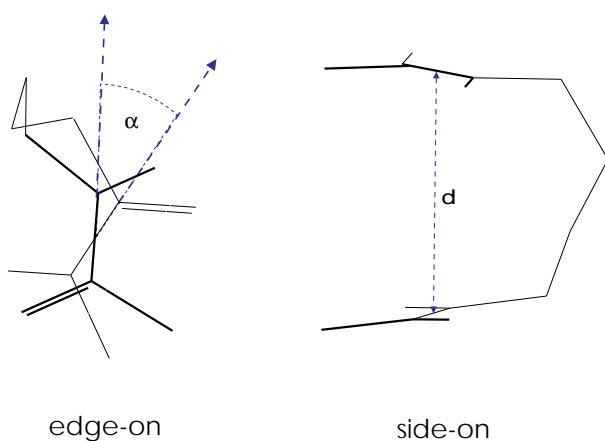
edge-on   side-on

Figure 12. *Wire graphic of the geometry of the amide-amide pi-complex in NAM-MEAM conformer 3 (PM3). Other atoms not belonging to the complex are omitted. The complex is shown edge-on (left) and side-on (right). The bonding parameters are: α = 30°; d= 3.85 A.*

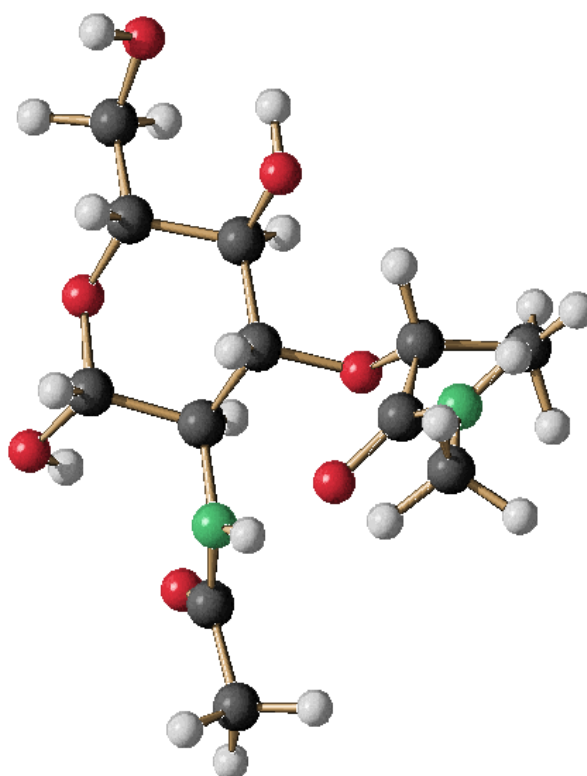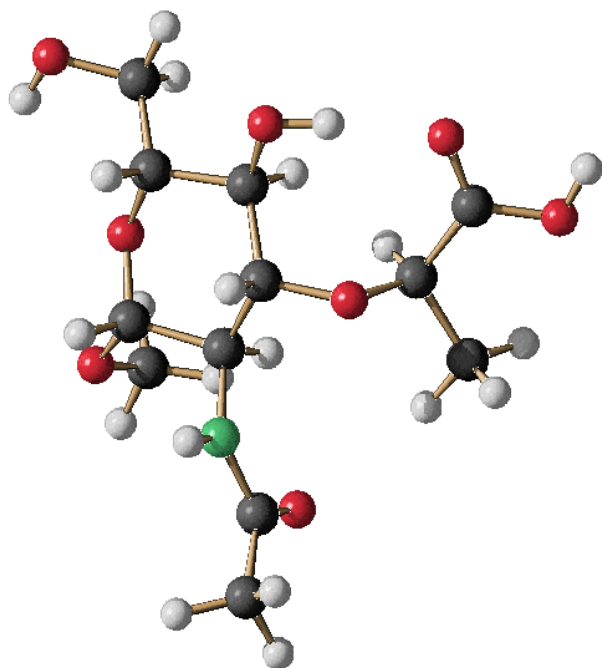**Figure 13.** *Molecular graphic of NAM-MEAM conformer 1', the energy minimum conformer by AM1 methode.*

Figure 14. *Molecular graphic of NAM-O1ME conformer 1, the energy minimum conformer calculated by PM3 method with amide correction potential.*

Figure 15. *Molecular graphic of NAM-O1ME conformer 1', the lowest energy conformer by AM1 methode.*

In the following, co-crystallizations of muramyl compounds with proteins, reported in the literature, will be discussed. Y. Bourne et al. [20] achieved the crystallization of muramic acid (not N-acetylated) and muramyl dipeptide in (D,D) configuration, complexed to a legume lectin and determined the X-ray structures. The ether torsional angles ($\theta 2$= 148°, $\theta 7$= 105°) of the muramyl dipeptide residue are situated in the near of region II (figure3). The amide plane of the N-acetyl sidechain is in cisoid position ($\theta 1$= 124°). The hydroxymethyl torsional angle ($\theta 4$ = -41°) is found in an energetically unfavourable position, bond C6-O6 gauche to C5-O5 and to C5-C4. The same position of the hydroxy-methyl group is found in the co-crystallized muramic acid structure. This might be due to a hydrogen bond between O6 and an Asp residue in the peptide chain, reported be Y. Bourne et al. [20] For the isolated NAM molecule, calculated in this study, such a double gauche position of the hydroxymethyl group is energetically strongly disfavoured. Atomic coordinates for the calculation of torsional angles of the compounds described by Y. Bourne et al. [20] have been taken from the Protein Data Bank, Brookhaven National Laboratory, Upton, NY, files 1loc.brk and 1lod.brk. The $\beta(1\rightarrow4)$ linked NAM-NAG-NAM trisaccharide (NAG: N-acetyl-2-Glucos-amine) bound to active-site cleft of hen egg-white lysozyme has been crystallographically studied by N.C.J. Strynadka and M.N.G. James [21]. The B-site NAM was less distorted. The experi-
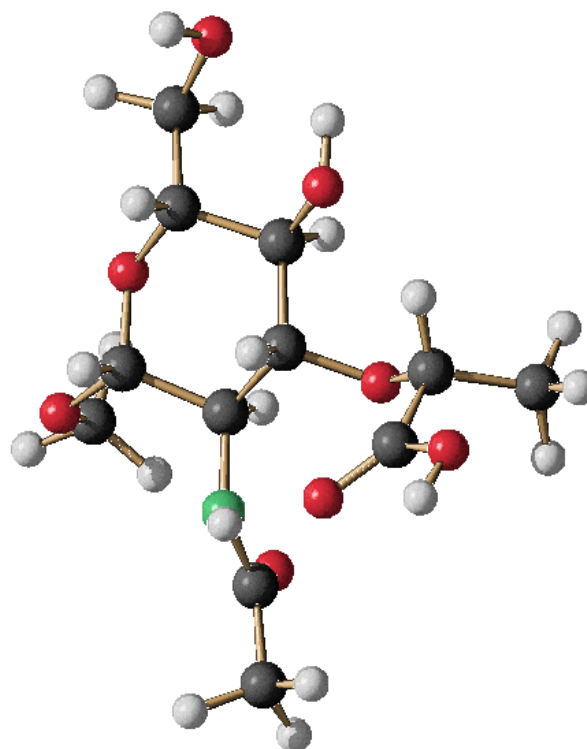
mental torsional angle value for the N-acetyl group was reported as $\theta 1$= 114°. The same value for $\theta 1$ has been calculated for the NAM conformer 3 using PM3 method (table 2).

C6 O-acetylated β-N-acetyl Muramic acid. The O-acetylation of the NAM residue may have certain biological significance according to the experimental results of S. Gyorffy and A.J. Clarke [23], who investigated the localization of O-acetylation of the P. mirabilis peptidoglycan by immunoelectron microscopy. W.B. Schweitzer and J.D. Dunitz [24] found conformational preferences in the average geometries of carboxylic esters by analysing data from many crystal structures retrieved from a database. For esters of primary alcohols usually the C-C bond is antiperiplanar to the ester C=O bond. In the nomenclature used in this study, $\theta 10$ (C-O-C=O) would be close to 0°. Remarkably, this statistically predicted ester geometry has been calculated for all low-energy NAM-O6AC conformers using the AM1 method, $\theta 10$ ranging between -4° and +5°.

Methylamide of β-N-acetyl Muramic acid. PM3 results showed a concurrence between a chained hydrogen bond geometry, conformer 1, and an amide-amide π-complex, conformer 3. In order to get an estimation of the amide-amide π-complex binding energy PM3 energy has been calculated for an analog complex consisting of two methyl-acetamide molecules in antiparallel orientation. We calculated a π-complex stabilization energy (PM3) of the dimer of 4.74 kcal/

mol. This is quite close to the stabilization energy (PM3) of one to two hydrogen bonds [13]. The methyl-acetamide dimer has a C=N midpoint-to-midpoint distance of d= 3.81 Å, only 0.04 Å shorter than the intramolecular amide-amide π-complex of NAM-MEAM conformer 3 (PM3). The twist angle α (pseudo-torsional angle N-midpoint-midpoint-C) was 0° for the dimer. Such a complex seems to be a real energetic alternative for the diamide compound according to PM3 calculations. AM1 gave no stable amide-amide π complex for the methyl-acetamide dimer, instead minimization led to a hydrogen bonded structure with a stabilization energy of 4.24 kcal/mol. This is a remarkable difference between AM1 and PM3 methods applied on amide-amide structures.

AM1 and PM3 proved to give reasonable molecular conformations for biomolecular structures like the saccharides calculated in this study. However, there are some differences in the hydrogen bond patterns between PM3 and AM1 low energy conformers. Because of the importance of muramyl-peptides as possible supporting treatment of cancer [25] and HIV-infections [26] a further semiempirical study on muramyl-monopeptide and an ab initio study on the peptide part of muramyl-dipeptide are in progress.

Conclusions

β-N-acetyl-Muramic acid - The amide plane of the N-acetyl group is either positioned strictly cisoid, or, in the occurence of one or two possible hydrogen bonds to neighboring side-chains, slightly rotated out of cisoid position by about 50° in clockwise direction, when viewed from the moving sidechain towards the fixed pyranosyl ring. - The torsional flexibility of the ether bridge of the lactyl group is sterically restricted to at least two well defined torsional angle regions I: $\theta 2$= -60° +/-15°, $\theta 7$= 120° +/- 30° II: $\theta 2$= 90° +/-20°, $\theta 7$= 90° +/- 30° - PM3 strongly favours the gauche position (C6-O6 in respect to C5-O5) of the hydroxymethyl group, whereas AM1 allows gauche and trans positions.

C6-O-acetylated β-N-acetyl-Muramic acid - The AM1 calculated cis geometry for the ester torsional angle (C-O-C=O) (= $\theta 10$) is in good agreement with the statistical average for esters of primary alcohols. - The torsional angle distribution at C6 of the acetylated sidechain shows no sterical preference according to both methods applied.

Methylamide of β-N-acetyl-Muramic acid - PM3 results slightly favour a chained hydrogen bonding structure O6-H6...O4-H4...O9 (O9 = lactyl-carbonyl oxygen) in concurrence to an amide-amide π complex with a distance of 3.85 Å.- AM1 predicts a hydrogen bonded structure between the two amide groups in the molecule.

Methylglycoside of β-N-acetyl-Muramic acid - PM3 favours a value of 70° for O5-C1-O1-C(aglycan) = $\theta 6$. AM1 strongly favours the values of 64° +/- 2°. - Overall structure of the energy minimum conformers practically uneffected by the introduction of the methyl group at O1.

Supplementary Material The molecular structures of the calculated energy minimum conformers are available as supplementary material in PDB-file format.

References

1. Barnickel, G.; Naumann, D.; Bradaczek, H.; Labischinski, H.; Giesbrecht , P. International FEMS Symposium on the Murein Sacculus of Bacterial Cell Walls; Hakenbeck, R., Hoeltje, J.-V., Labischinski, H.(Eds.) Walter de Gruyter: Berlin, New York, 1983; pp 61-66.
2. Yadav, J.S.; Labischinski, H.; Barnickel, G.; Bradaczek, H. *J. Theor. Biol.* **1981**, *88*, 441
3. Yadav, J.S.; Barnickel, G.; Bradaczek, H. *J. Theor. Biol.* **1982**, *95*, 151
4. Yadav, P.N.S.; Rai, D.K.; Yadav, J.S. *J. Mol. Struct.* **1989**, *194*, 19
5. Rogers, J.H.; Perkins, H.R.; Ward, J.B. Microbial Cell Walls and Membranes; Chapman & Hall: London, 1980
6. Weidel, W.; Pelzer, H. *Adv. Enzymol.* **1964**, *26*, 193
7. Fleck, J.; Mock, M.; Minck, R.; Ghuysen, J.-M. *Biochim. Biophys. Acta.* **1971**, *233*, 489
8. Perkins, H.R.; Chapman, S.J.; Blundell, J.K. International FEMS Symposium on the Murein Sacculus of Bacterial Cell Walls; Hakenbeck, R., Hoeltje, J.-V., Labischinski, H. (Eds.) Walter de Gruyter: Berlin, New York, 1983; pp 255-260.
9. Burghaus, P.; Johannsen, L.; Naumann, D.; Labischinski, H.; Bradaczek, H.; Giesbracht, P. International FEMS Symposium on the Murein Sacculus of Bacterial Cell Walls; Hakenbeck, R., Hoeltje, J.-V., Labischinski, H. (Eds.) Walter de Gruyter: Berlin, New York, 1983; pp 317-322.
10. Clarke, A.J.; Dupont, C. *Can. J. Microbiol.* **1992**, *38*, 85.
11. Johannsen, L.; Labischinski, H.; Burghaus, P.; Giesbrecht, P. International FEMS Symposium on the Murein Sacculus of Bacterial Cell Walls; Hakenbeck, R., Hoeltje, J.-V., Labischinski, H. (Eds.) Walter de Gruyter: Berlin, New York, 1983; pp 261-266.
12. Johannsen, L. *APMIS* **1993**, *101*, 337.
13. Jurema, M.W.; Shields, G.C. *J. Comput. Chem.* **1993**, *14*, 89.
14. Khalil, M.; Woods, R.J.; Weaver, D.F.; Smith, Jr. V.H. *J. Comput. Chem.* **1991**, *12*, 584.
15. Scano, P.; Thompson, C. *J. Comput. Chem.* **1991**, *12*, 172.
16. Stewart, J.J.P. *J. Comput. Chem.* **1989**, *10*, 209.
17. Dewar, M.J.S.; Zoebisch, E.G.; Healy, E.F.; Stewart, J.J.P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
18. Knox, J.R.; Murthy, N.S. *Acta Cryst.* **1974**, *B30*, 365.
19. Mo, F. *Acta Chem. Scand. Ser. A* **1979**, *33*, 207.
20. Bourne, Y.; Ayouba, A.; Rouge, P.; Cambillau, C. *J. Biol. Chem.* **1994**, *269*, 9429.

21. Strynadka, N.C.J., James, M.N.G. *J. Mol. Biol.* **1991,** *220*, 401.

22. Pincus, M.R.; Burgess, A.W.; Scheraga, H.A. *Biopolymers* **1976,** *15*, 2485.

23. Gyorffy, S.; Clarke, A.J. *J. Bacteriol.* **1992,** *174*, 5043.

24. Schweizer, W.B.; Dunitz, J. D. *Helv. Chim. Acta.* **1982,** *65*, 1547

25. Zidek, Z.; Frankova, D. *Int. J. Immunopharmacol.* **1995,** *17*, 313

26. Maruyama, Y.; Kurimura, M.; Achiwa, K. *Chem. Pharm. Bull.* **1994,** *42*, 1709.