

## 194. D-Gluconhydroximo-1,5-lactam and Related N-Arylcarbamates

### Theoretical Calculations, Structure, Synthesis, and Inhibitory Effect on $\beta$ -Glucosidases

by Roland Hoos<sup>1)</sup>, Andrew B. Naughton<sup>2)</sup>, Walter Thiel, Andrea Vasella<sup>1)\*</sup>,  
and Wolfgang Weber

Organisch-Chemisches Institut der Universität Zürich, Winterthurerstr. 190, CH-8057 Zürich

and Karen Rupitz, and Stephen G. Withers

Department of Chemistry, The University of British Columbia,  
2036 Main Mall, Vancouver, B.C., V6T 1Z1 Canada

(17.VIII.1993)

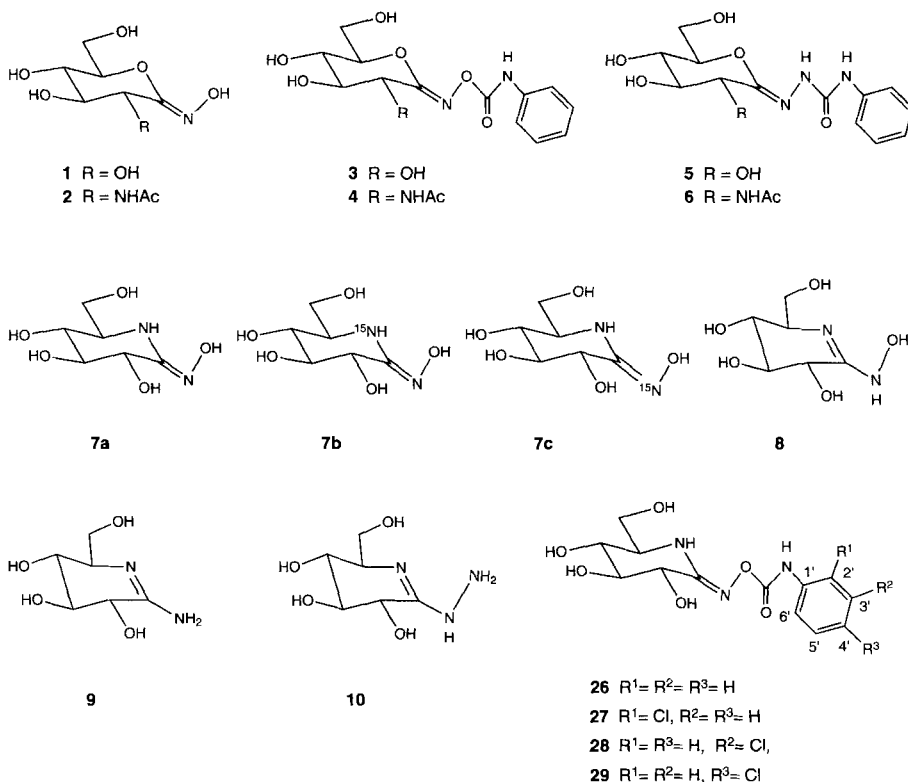
The known D-gluconhydroximo-1,5-lactam (= D-glucono-1,5-lactam oxime) **7a**, its nitrogen isotopomers **7b** and **7c**, and the N-arylcarbamates **26–29** were synthesized from 2,3,4,6-tetra-O-benzyl-D-glucono-1,5-lactam (**11a**) and its nitrogen isotopomer **11b** to establish the controversial structure of **7a** and to study the inhibition of  $\beta$ -glucosidases by the N-arylcarbamates **26–29**. Conversion of **11a** with Lawesson's reagent yielded a mixture of the thionolactam **15a** and its *manno*-configured isomer **16a**, which was transformed into a mixture of the benzylated hydroximo-lactam **13a** and the *manno*-isomer **17a**. Debenzylation (Na/NH<sub>3</sub>) and acetylation of this mixture led to the *gluco*-configured pentaacetate **14a** and the *manno*-isomer **18a**. Treatment of **11a** with Et<sub>3</sub>O·BF<sub>4</sub> and then with H<sub>2</sub>NOH gave exclusively the benzylated D-gluconhydroximo-1,5-lactam (benzylated D-nojirilactam oxime) **13a**, which was transformed into **14a**. Deacetylation of **14a** yielded the hydroximo-lactam **7a**. The isotopomers **7b** and **7c** were obtained by analogous reaction sequences, using either <sup>15</sup>NH<sub>3</sub> or <sup>15</sup>NH<sub>2</sub>OHHCl. To prepare the acetylated N-arylcarbamates **20–25**, **13a** was debenzylated and acetylated ( $\rightarrow$  **14a**), followed by selective deacetylation to the tetraacetate **19a** and treatment with the appropriate isocyanates. The structure of the 2-chlorophenyl carbamate **21** was established by X-ray analysis. Deacetylation of **20–23** led to the N-arylcarbamates **26–29**.

The <sup>15</sup>N-NMR spectra of **7b**, **7c**, and of their precursors **13b**, **13c**, **14b**, and **14c**, show that the C=N bond in all these lactam oximes is exocyclic as predicted from semiempirical and *ab initio* SCF-MO calculations on the structure of acetamide oxime and 5-pentanelactam oxime. According to these calculations, 5-pentanelactam oxime is a (*Z*)-configured, flattened chair. X-ray analysis established the structure of D-glucono-1,5-lactam oxime (**7a**) in the solid state, where it adopts a conformation between <sup>4</sup>C<sub>1</sub> and <sup>4</sup>H<sub>3</sub>. In H<sub>2</sub>O, **7a** is a flattened <sup>4</sup>C<sub>1</sub>. The calculations also predict that protonation at the exocyclic N-atom strengthens the conjugation between the endocyclic N-atom and the hydroxyimino group, and leads to a half-chair conformation. This is evidenced by the chemical shift differences in the <sup>15</sup>N-NMR spectra observed upon protonation of **7b** and **7c**. The hydroximo-lactam **7a** and the N-arylcarbamates **26–29** are competitive inhibitors of the  $\beta$ -glucosidases from sweet almond (emulsin) and from *Agrobacterium faecalis* (= *Abg*), with *K<sub>i</sub>* values between 8 and 21·10<sup>-6</sup> M against emulsin (at pH 6.8) and between 0.15 and 1.2·10<sup>-6</sup> M against *Abg* (at pH 7.0).

<sup>1)</sup> Present address: Laboratorium für Organische Chemie, ETH-Zentrum, Universitätsstrasse 16, CH-8092 Zürich.

<sup>2)</sup> Present address: Lonza, Riverside, 900 River Road, Conshohocken, PA 19428, USA.

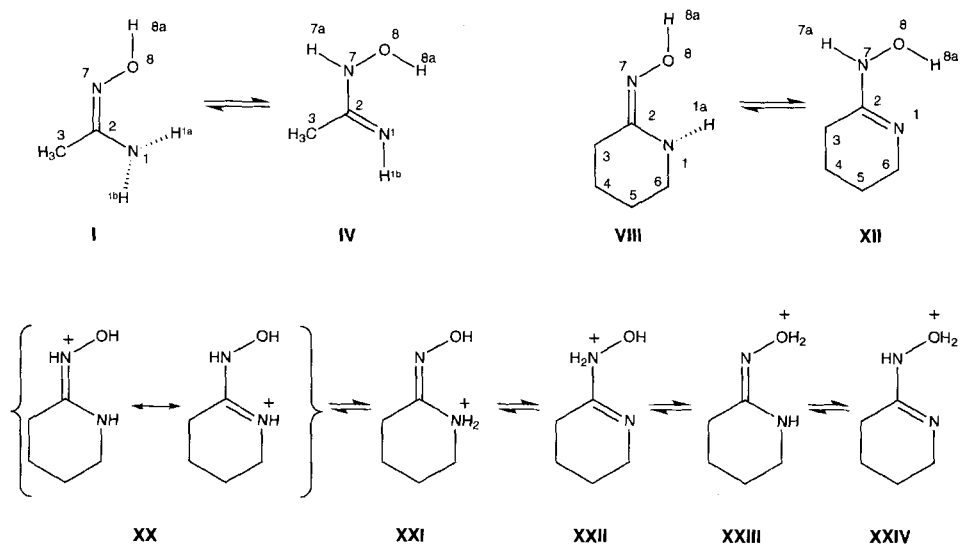
**Introduction.** – The hydroximo-lactones (= lactone oximes) **1** [1] and **2** [2], the corresponding *N*-phenylcarbamates **3** [1] and **4** [2], and the semicarbazones **5** and **6** [3] are strong ( $K_1$  between  $10^{-5}$  and  $10^{-8}$  M), competitive, and neutral glycosidase inhibitors, suggesting that the related 5-amino-5-deoxy and 5-thio-5-deoxy analogues should also be prepared and evaluated as glycosidase inhibitors. *Ganem* and *Papandreou* [4] have, indeed, reported on the synthesis and enzymatic testing of the parent hydroxyamino lactam (= lactam oxime) **8**, and the related amidine **9** and amidrazone **10**, which they classified as transition-state analogues, and as broad-spectrum inhibitors. The inhibitory properties were traced back primarily to the shape, rather than to the basic character of **8–10**. Their shape, and particularly their conformation, were considered to be a consequence of the proposed constitution **8**, *i. e.* of the endocyclic C=N bond, and the hydroximo-lactam was claimed to be the first neutral inhibitor possessing a well-defined half-chair conformation. We have already [5] formulated doubts about the proposed constitution of the hydroximo-lactam, which should be **7** rather than **8**. This is suggested by X-ray data of related amide oximes as available from the *Cambridge Data Files*, and by NMR studies of amide oximes<sup>3)</sup> (*see* [6] and *lit. cit. there*).



<sup>3)</sup> Similarly, the structure of a hydrazino-imine was postulated for the amidrazone **10**. This is at variance with the structure of the semicarbazone **6** [3], the X-ray analysis of which strongly suggests the presence of an exocyclic, (*Z*)-configured C=N bond. The constitution of this amidrazone remains to be established.

We now report the results of calculations of the relative stability of amide and lactam oximes and their (hydroxyamino)imine tautomers, the synthesis of D-gluconhydroximo-1,5-lactam (= D-glucono-1,5-lactam oxime) **7a**<sup>4</sup>) and its nitrogen isotopomers **7b** and **7c**, the proof of their constitution, the preparation of the *N*-arylcarbamates **26–29**, and their properties as glucosidase inhibitors.

**Results and Discussion.** – 1. *Quantum-Chemical Calculations.* We carried out semiempirical and *ab initio* SCF-MO calculations on acetamide oxime, 5-pentanelactam oxime, and their tautomers. Molecular geometries were fully optimized without any symmetry constraints. The AM1 method [7] was used for systematic conformational searches. We found three minima (**I–III**) for acetamide oxime, four minima (**IV–VII**) for 2-(hydroxyamino)ethanimine, four chair (**VIII–XI**) and two twist (**VIIIa, VIIIb**) conformers for 5-pentanelactam oxime, and eight half-chair conformations (**XII–XIX**) for 2,3,4,5-tetrahydro-2-(hydroxyamino)pyridine. *Fig. 1* depicts the most stable of these species (*i. e.* **I, IV, VIII, and XII**), and defines the relevant dihedral angles. The five tautomers (**XX–XXIV**) considered for the protonated cyclic compounds were derived from the most stable neutral conformers (*i. e.* from **VIII** and **XII**). Force constant analyses confirmed that each of the optimized AM1 structures (**I–XXIV**) is a minimum on the corresponding potential surface.



*Fig. 1. Survey of Tautomers of Acetamide Oxime and 5-Pentanelactam Oxime.* Only the most stable AM1 structure is indicated for conformers **I–III**, **IV–VII**, **VIII–XI**, and **XII–XIX**. The numbering in **I, IV, XX–XXIV** is analogous to that in **VIII** and **XII**. Dihedral angles:  $\alpha = \text{H}(1b)\text{--N}(1)\text{--C}(2)\text{--C}(3)$  in **I–VII**,  $\alpha = \text{C}(6)\text{--N}(1)\text{--C}(2)\text{--C}(3)$  otherwise;  $\beta = \text{N}(1)\text{--C}(2)\text{--N}(7)\text{--O}(8)$ ;  $\gamma = \text{C}(2)\text{--N}(7)\text{--O}(8)\text{--H}(8a)$ ;  $\delta = \text{H}(1a)\text{--N}(1)\text{--C}(2)\text{--N}(7)$  in **I–III**, **VIII–IX**, **XXI**, and **XXIII**, and  $\delta = \text{N}(1)\text{--C}(2)\text{--N}(7)\text{--H}(7a)$  in **IV–VII**, **XII–XIX**, **XXII**, and **XXIV**.

<sup>4</sup>) Throughout the text and *Schemes*, **a** corresponds to compounds with the natural distribution of N isotopes, **b** to <sup>15</sup>N–C(5) (= endocyclic N)-labeled, and **c** to <sup>15</sup>N–C(1) (= exocyclic N)-labeled isotopomers.

Table 1. Relative Energies [kcal/mol]<sup>a)</sup> and Selected Dihedral Angles [°]<sup>b)</sup>.

Species	$\Delta\Delta H_f$	$\Delta E_{tot}$	$\alpha$	$\beta$	$\gamma$	$\delta$	Analogue
<b>I</b>	0.0	0.0	32	6	-177	-17	
<b>II</b>	3.5	3.9	40	-174	175	12	
<b>III</b>	6.5		49	-173	-5	-6	
<b>IV</b>	11.7	10.3	1	-25	78	-143	
<b>V</b>	12.0	9.4	4	-138	-100	-19	
<b>VI</b>	15.2		-2	-18	53	106	
<b>VII</b>	16.1		3	-131	67	-6	
<b>VIII</b>	0.0	0.0	40	6	-177	-11	<b>I</b>
<b>VIIIa<sup>c)</sup></b>	1.8	3.2	22	5	-178	-21	
<b>VIIIb<sup>c)</sup></b>	1.3		18	5	-178	-29	
<b>IX</b>	3.4	3.2	43	-173	177	-9	<b>II</b>
<b>X</b>	3.4		46	-2	9	103	
<b>XI</b>	7.0		46	-171	-6	-7	<b>III</b>
<b>XII</b>	6.89	10.1	-2	25	-78	142	<b>IV</b>
<b>XIII</b>	6.94		0	-23	79	-141	<b>IV</b>
<b>XIV</b>	7.28	7.7	3	-136	-102	-17	<b>V</b>
<b>XV</b>	7.34		-5	135	101	18	<b>V</b>
<b>XVI</b>	10.39		-3	-18	53	107	<b>VI</b>
<b>XVII</b>	10.23		1	20	-54	-101	<b>VI</b>
<b>XVIII</b>	11.06		2	-129	66	-4	<b>VII</b>
<b>XIX</b>	11.33		-4	134	-65	9	<b>VII</b>
<b>XX<sup>d)</sup></b>	0.0	0.0	-6	18	-121	<sup>e)</sup>	
<b>XXI</b>	7.8	21.6	54	0	180	-4/115	
<b>XXII</b>	28.2	34.2	-1	56	-53	-69/173	
<b>XXIII</b>	38.7	38.1	-6	-1		2	
<b>XXIV</b>	56.8		-1	11		127	

<sup>a)</sup>  $\Delta\Delta H_f$  from differences of AM1 heats of formation,  $\Delta E_{tot}$  from differences of *ab initio* total energies (6-31G\* SCF).

<sup>b)</sup> AM1 values, notation see *Fig. 1* ( $\alpha, \beta, \gamma, \delta$ ). <sup>c)</sup> Maximum dihedral angle ( $\approx 57^\circ$ ) in the ring: **VIIIa** C(2)-C(3)-C(4)-C(5), **VIIIb** N(1)-C(2)-C(3)-C(4). <sup>d)</sup> Proton affinities for **VIII** + H<sup>+</sup> → **XX**. a) AM1: -219.7 kcal/mol (from heats of formation following the recommended procedure [37]); b) 6-31G\* SCF: -245.9 kcal/mol (from total energies).

<sup>e)</sup> H(1)-N(1)-C(2)-N(7): 150°, N(1)-C(2)-N(7)-H(7a): -7°.

*Ab initio* SCF-MO calculations were performed for selected conformers using the 6-31G\* basis set [8] [9] and the TURBOMOLE program [10]. The optimized AM1 structures served as starting points for the *ab initio* geometry optimizations.

*Table 1* summarizes the main results. In almost all cases where such comparisons are possible, the AM1 and *ab initio* calculations yield qualitatively the same types of conformers, with deviations in the relevant dihedral angles of normally less than 5°. Larger deviations which, however, are not important qualitatively occur only for the imines **IV** and **XII** (particularly with regard to  $\gamma$ ). For the sake of brevity, *Table 1* lists only the AM1 dihedral angles. Both the AM1 and *ab initio* calculations identify **I**, **VIII**, and **XX** as the most stable species on the three potential surfaces studied, and predict rather similar relative energies for the other conformers and tautomers (with the exception

of **XXI**). Overall, the AM1 and *ab initio* results for the geometries and relative energies agree quite well with each other. In the following, we comment on some specific results in Table 1.

In the oxime conformers of *acetamide oxime*, all heavy atoms lie almost in one plane. The conformational degrees of freedom are associated with rotations around the N(1)–C(2), C(2)–N(7), and N(7)–O(8) bonds (*i. e.* dihedral angles  $\alpha$ ,  $\beta$ , and  $\gamma$ , respectively, see Fig. 1). The NH<sub>2</sub> group is nonplanar and always adopts one particular orientation with respect to the N(1)–C(2)–N(7) plane ( $\alpha \approx 32\text{--}49^\circ$ ,  $-\delta \approx 6\text{--}17^\circ$  in **I–III**, see Table 1). With regard to rotations around the C(2)–N(7) and N(7)–O(8) bonds, the *cis/trans*-(**I**), *trans/trans*-(**II**), and *trans/cis*-(**III**) conformers are found to be local minima on the AM1 potential surface, whereas there is no minimum for the *cis/cis*-structure. The most stable form of the oxime is **I**, both at the AM1 and the *ab initio* level<sup>5</sup>). Changing the configuration at the C=N bond from (*Z*) (**I**) to (*E*) (**II**) increases the energy by 3.5 (AM1) and 3.9 (*ab initio*) kcal/mol, respectively. The preference for the (*Z*)-configuration in **I** is consistent with the crystal structure of formamide oxime [12], where *ab initio* calculations predict a (*Z*) vs. (*E*) stabilization energy of 3.7 kcal/mol [13].

Only structures with (*E*)-configured N(1)=C(2) bond of 2-(*hydroxyamino*)ethanimine were taken into account, since only this configuration is sterically accessible to the cyclic compounds **XII–XIX** (see below). The imine conformers are essentially planar around the N(1)=C(2) bond and nonplanar at N(7). There are four local minima (**IV–VII**) on the AM1 potential surface. Both the N(7)–O(8) and N(7)–H(7a) bonds can be approximately *cis* (eclipsed) to the N(1)=C(2) bond, and in each of these cases the second substituent at N(7) can assume two different orientations (see Table 1). The preferred conformers **IV** and **V** are calculated to be within a range of 1 kcal/mol, with reverse order at the AM1 and *ab initio* level. More important, however, is the prediction that all imine conformers (**IV–VII**) are higher in energy than the oxime conformers (**I–III**), both by the AM1 and *ab initio* calculations. The energy difference between the most stable oxime and imine conformers is 11.7 (AM1) and 9.4 (*ab initio*) kcal/mol, respectively<sup>6</sup>).

There are eight possible chair conformations for the saturated six-membered ring of 5-pentanelactam oxime, which are distinguished by the configuration of the C(2)=N(7) bond (*Z* or *E*), of the N(7)–O(8) bond (*s-cis* or *s-trans*), and at N(1) (equatorial or axial substituent H(1a), ring inversion). On the AM1 potential surface, there are *cis/trans* (**VIII**), *trans/trans* (**IX**), and *trans/cis* (**XI**) minima with equatorial H(1a), completely analogous to the corresponding acetamide oximes **I–III** with regard to both relative energies and geometries (see Table 1). At the AM1 level, there is also a *cis/cis* (**X**) minimum with axial H(1a), which disappears at the *ab initio* level. The remaining four conformers collapse upon geometry optimization to one of the minima (**VIII–XI**). The

<sup>5</sup>) Previous *ab initio* studies [11] have considered only a C<sub>s</sub> structure for acetamide oxime with a planar amino group which lies 0.55 kcal/mol above the C<sub>1</sub> minimum (*ab initio*).

<sup>6</sup>) To be complete, we have also located several minima for the nitroso and nitrono tautomers of acetamide oxime on the AM1 surface. The nitroso and nitrono conformers are calculated to be higher in energy than the most stable oxime **I**, by 5.9–8.1 and 17.6–20.3 kcal/mol, respectively. According to previous *ab initio* studies on nitrosomethane [14], these tautomers are separated by large barriers from the corresponding oximes. They should not be relevant to the present experimental work.

chairs in **VIII–XI** are slightly flattened ( $\alpha = 40\text{--}46^\circ$ ), and the orientation of the bonds at N(1) is surprisingly close to that in the oximes **I–III** ( $\alpha = 32\text{--}49^\circ$ , see *Table 1*). Keeping the favored *cis/trans* oxime conformation from **VIII**, we have also located two AM1 minima (**VIIIa**, **VIIIb**) with a twist conformation in the six-membered ring which lie slightly above the chair conformer **VIII** in energy, as expected. Re-optimization of **VIIIa** at the *ab initio* level leads to very minor changes in geometry and to a slight increase in the relative energy. Based on these results (see *Table 1*), we conclude that **VIII** is the most stable conformer of the cyclic oxime.

The *2,3,4,5-tetrahydro-2-(hydroxyamino)pyridine* tautomer adopts only half-chair conformations (**XII–XIX**), since the N(1)=C(2) bond and the adjacent atoms (C(3), C(6), and N(7)) are approximately coplanar. Inversion of the half-chair leads to two conformations for each of the minima encountered for 2-(hydroxyamino)ethanimine (**IV–VII**), so that there are four pairs of conformers (**XII/XIII**, **XIV/XV**, **XVI/XVII**, **XVIII/XIX**). The conformers within a pair are structurally and energetically very similar. All conformers **XII–XIX** are significantly higher in energy than the most stable oxime **VIII**, in analogy to the acyclic case (**I–VII**, see *Table 1*). The energy difference between the most stable conformers of either of the tautomers is 6.9 (AM1) and 7.7 (*ab initio*) kcal/mol, respectively.

*Protonation* of the cyclic compounds **VIII–XIX** can occur at N(1), N(7), or O(8). AM1 calculations on many of the resulting protonated species indicate that the relative energies of the various conformers are quite similar to those of the corresponding neutral molecules. Therefore, we only discuss the protonated tautomers **XX–XXIV** which are derived from the most stable neutral conformers **VIII** and **XII**. The lowest-energy tautomer **XX** can be regarded as **VIII** protonated at N(7) or as **XII** protonated at N(1). Judging from the dihedral angles ( $\alpha = -6^\circ$ ,  $\beta = 18^\circ$ , see *Table 1*), the latter description is more adequate: **XX** adopts a half-chair conformation similar to **XII**. The second most stable tautomer **XXI** assumes a chair configuration ( $\alpha = 54^\circ$ ) with an exocyclic double bond ( $\beta = 0^\circ$ ) and thus resembles **VIII**. This is also evident from the optimized bond lengths (see *Table 2*) which show a strong alternation between N(1)–C(2) and C(2)–N(7) that is even more pronounced in **XXI** than in **VIII**. As expected for an allyl-type system, there is little bond-length alternation in **XX**, N(1)–C(2) being slightly shorter than C(2)–N(7). The remaining tautomers **XXII–XXIV** are considerably higher in energy (see *Table 1*).

Table 2. Selected Bond Lengths [Å] at the 6-31G\* SCF Level<sup>a)</sup>b)

Species	N(1)–C(2)	C(2)–N(7)
<b>I</b>	1.371	1.262
<b>IV</b>	1.257	1.385
<b>VIII</b>	1.374	1.263
<b>XII</b>	1.249	1.397
<b>XX</b>	1.292	1.328
<b>XXI</b>	1.477	1.245

<sup>a)</sup> For comparison [38]: H<sub>3</sub>C–NH<sub>2</sub> 1.453 Å (exp.: 1.471 Å), H<sub>2</sub>C=NH 1.250 Å (exp.: 1.273 Å). <sup>b)</sup> At the AM1 level, the bonds are consistently longer by ca. 0.02–0.06 Å, but the trends are the same.

Thus, according to the quantum-chemical calculations, the acyclic (**I–VII**) and cyclic (**VIII–XIX**) model compounds are quite similar with respect to the preferred tautomers and conformers and with regard to structural and energetic details. The oxime form is favored for the neutral species. The most stable conformer (**VIII**) of 5-pentanelactam oxime is a slightly flattened chair with (*Z*)-configuration around the exocyclic C(2)=N(7) bond. Protonation of **VIII** leads to a considerable change in geometry, however, since the most stable tautomer **XX** is best described as a protonated imine (derived from **XII**), with a half-chair conformation and a pyramidal exocyclic N(7)-atom.

2. *Synthesis and Structure of D-Gluconhydroximo-1,5-lactam (= D-Glucono-1,5-lactam Oxime, 7a), Its Nitrogen Isotopomers 7b and 7c, and the N-Arylcarbamates 26–29.* To establish the structure of **7** without having to resort to X-ray analysis, we intended to prepare the <sup>15</sup>N-labeled isotopomers **7b** and **7c**. The <sup>15</sup>N chemical shift and the <sup>1</sup>H,<sup>15</sup>N coupling of **7b** or **7c** should allow an unambiguous determination of the constitution of **7a**, which has been prepared by *Ganem* and coworkers from nojirilactam [15]. The synthesis of nojirilactam from nojirimycin proceeds in ca. 20% yield [16]<sup>7)</sup>, but the aim of preparing both monolabeled <sup>15</sup>N isotopomers of **7a** and the *N*-arylcarbamate derivatives **26–29**, and the anticipated versatility of protected derivatives of nojirilactam<sup>8)</sup> as synthetic intermediates has prompted us to develop a large-scale synthesis of 2,3,4,6-tetra-*O*-benzyl-D-gluconolactam (**11a**, *Scheme 1*) [18]<sup>9)</sup> from 2,3,4,6-tetra-*O*-benzyl-D-glucose. The synthesis proceeds in an overall yield of 43%; it was easily adapted to the preparation of the <sup>15</sup>N-labeled isotopomer **11b** and further improved by using freshly prepared pyridine · SO<sub>3</sub> for the oxidation of 2,3,4,6-tetra-*O*-benzyl-D-(<sup>15</sup>N)-gluconamide (**30**), thus increasing the overall yield to 53%.

Activation of **11a** by *O*-alkylation, similarly to a procedure applied by *Ganem* and coworkers [15] to transform nojirilactam, yielded the imino ether **12a**, which, upon treatment with NH<sub>2</sub>OH, gave the benzylated hydroximo-lactam **13a** and hence, by *Birch* reduction [21] and acetylation, the pentaacetate **14a** in 30–40% overall yield. The same procedure transformed **11c** into **14c**. Activation of **14a** by thionation [22], again similarly to a procedure reported by *Ganem* and coworkers [15], proceeded in higher yields than *O*-alkylation, but gave an inseparable, crystalline 9:1 mixture of the *gluco*-configured thionolactam **15a** and an isomer, to which the *manno*-configuration **16a** was tentatively assigned. The mixture was treated with NH<sub>2</sub>OH to yield a 10:1 mixture of **13a** and **17a**. *Birch* reduction and acetylation of this mixture, followed by a tedious chromatography, yielded pure **14a** as the major product besides the *manno*-configured **18a**.

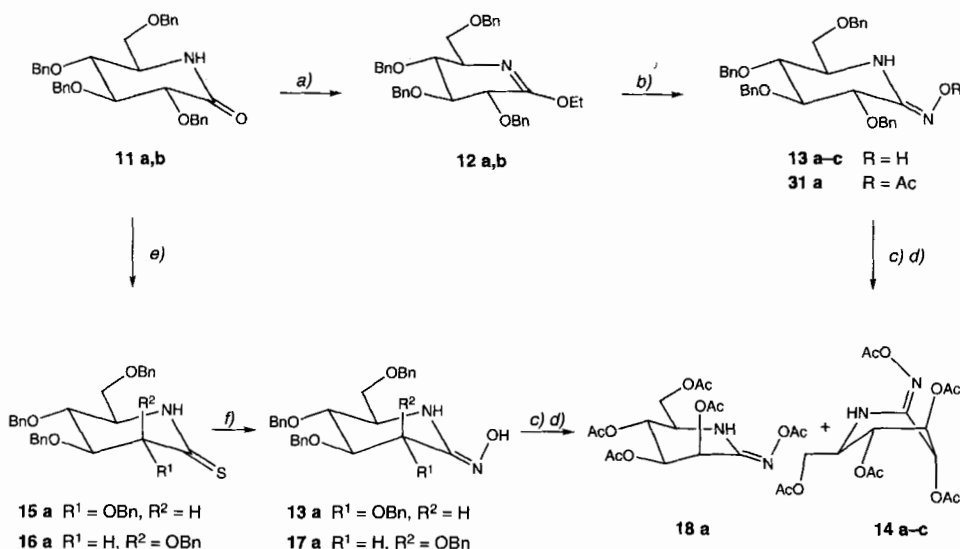
*O*-Alkylation of the gluconolactam **11a** is evidenced by the EtO signals at 1.12 (*t*, *J* ≈ 7.0 Hz) and at 4.18 ppm (*q*, *J* ≈ 7.0 Hz). The imino group of **12** gives rise to a C(1) *s* at 161.11 ppm, and to a sharp IR band at 1675 cm<sup>-1</sup>. The thionolactam **15a** is characterized by a *s* at 200.3 ppm, and a C=S band at 1545 cm<sup>-1</sup>. Compared to the

<sup>7)</sup> *Ganem* and coworkers [15] reported a modified procedure, but did not indicate the yield.

<sup>8)</sup> According to [15], the trimethylsilyl-protected nojirithionolactam, but not the acetylated nojirilactam (acetylated D-gluconolactam) is suitable for the preparation of **7**. Both compounds were derived from nojirimycin. 3,6-Di-*O*-benzyl-D-gluconolactam, described by *Fleet et al.* [17], was not suitable for our purpose.

<sup>9)</sup> The procedure is based upon a patent [19] but leads to substantially improved yields; a similar procedure has been reported by *Pandit* and coworkers [20].

Scheme 1



a)  $\text{Et}_3\text{O}\cdot\text{BF}_4$ ,  $\text{CH}_2\text{Cl}_2$ , r.t., 26 h,  $\text{Et}_3\text{N}$ ,  $0^\circ \rightarrow \text{r.t.}$ , 1 h; 50%. b)  $\text{NH}_2\text{OH}$ , MeOH, r.t., 40 min; 86%. c) Na,  $\text{NH}_3$ , THF, reflux, 15 min. d)  $\text{Ac}_2\text{O}$ , pyridine, DMAP, r.t., 2 h; 68–78% from **13a-c**. e) 2,4-Bis(4-methoxyphenyl)-2,4-dithioxo-1,3,2,4-dithiadiphosphetan (Lawesson's reagent),  $\text{C}_6\text{H}_6$ , reflux, 6 h; 99%. f)  $\text{NH}_2\text{OH}\cdot\text{HCl}$ ,  $\text{NaHCO}_3$ , MeOH, reflux, 2 h; 92%.

$^1\text{H-NMR}$  spectrum of the lactam **11a**, the NH and the H-C(2) signals are shifted downfield by 2.2 and 0.4 ppm. A second species **16a**, closely related to **15a**, was characterized by a second set of similar signals. The integration of the  $\text{PhCH}_2$  *d* of **15a** at 4.99 ppm and a corresponding *d* of **16a** at 5.05 ppm gives a ratio of 10:1 in favor of the *gluco*-isomer **15a**. The configuration of **15a** and of **16a** were deduced from those of **14a** and **16a** (see below), as the NMR spectra of **15a/16a** and of **13a/17a** were insufficiently well resolved for unambiguous configurational assignments.

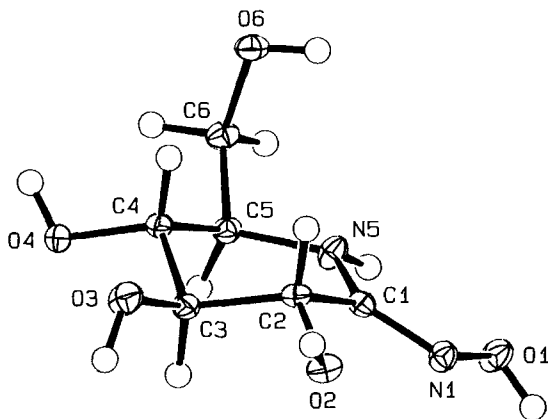
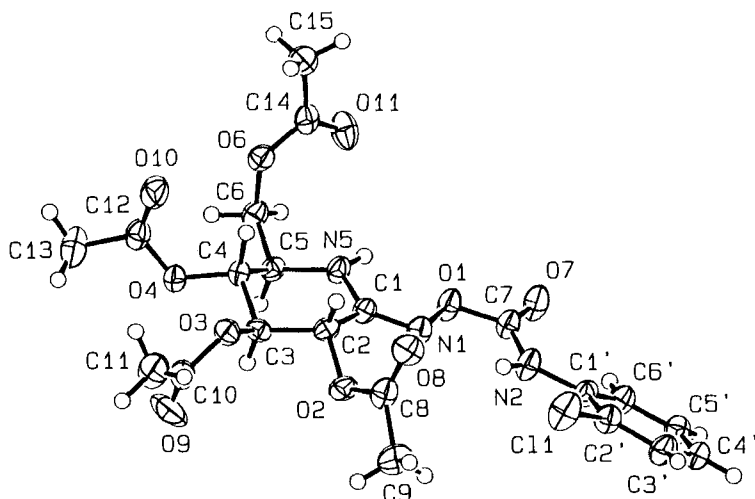
The benzylated lactam oxime **13a** is characterized by NH, OH, and C=N IR bands at 3620, 3430, and 1670  $\text{cm}^{-1}$ . Its structure is further evidenced by an exchangeable NH *s* at 5.46 ppm, and by a  $^{13}\text{C}$  *s* at 149.79 ppm. Acetylation of **13a** to **31a** shifted the C(1) signal downfield by only 3.03 ppm, evidencing the (*Z*)-configuration<sup>10)</sup>. This configuration is confirmed by the X-ray analyses of **7a** (Fig. 2) and of the acetylated 2-phenylcarbamate **21** (Fig. 3).

The *J* values in the  $^1\text{H-NMR}$  spectrum ( $\text{CDCl}_3$ ) of **14a** ( $J(2,3) = 4.5$ ,  $J(3,4) = 4.5$ ,  $J(4,5) = 9.5$  Hz) indicate a  $B_{2,5}$  conformation. A H-C(2)-NH *W* coupling of ca. 1.5 and 1.7 Hz is observed for both, **14a** and **18a**, respectively. It indicates a planar arrangement of C(2), C(1), and N(5) and is in keeping with a  $B_{2,5}$  conformation of the lactam oxime **14a**, and, together with  $J(2,3) = 3.3$ ,  $J(3,4) = 8.9$ , and  $J(4,5) = 7.5$  Hz, with a  $^4H_3$  conformation of the *manno*-configured **18a**.

Deacetylation (Scheme 2) gave **7a** from **14a**. The isotopomer **7b**, labeled at the endocyclic N-atom, was prepared from **11b** via **14b**, in close analogy to **7a**. The isotopomer **7c**, labeled at the exocyclic N-atom, was obtained by treating the pure imino

<sup>10)</sup> C(1) of tetra-*O*-benzyl-*D*-gluconhydroximo-1,5-lactone [23] resonates at 151.43 ppm. Upon acetylation, this signal is shifted downfield by 4.53 ppm. Upon diethylphosphorylation, this signal is shifted downfield by 5.9 ppm for the (*Z*)-, and by 17.9 ppm for the (*E*)-configured hydroximo-lactone.

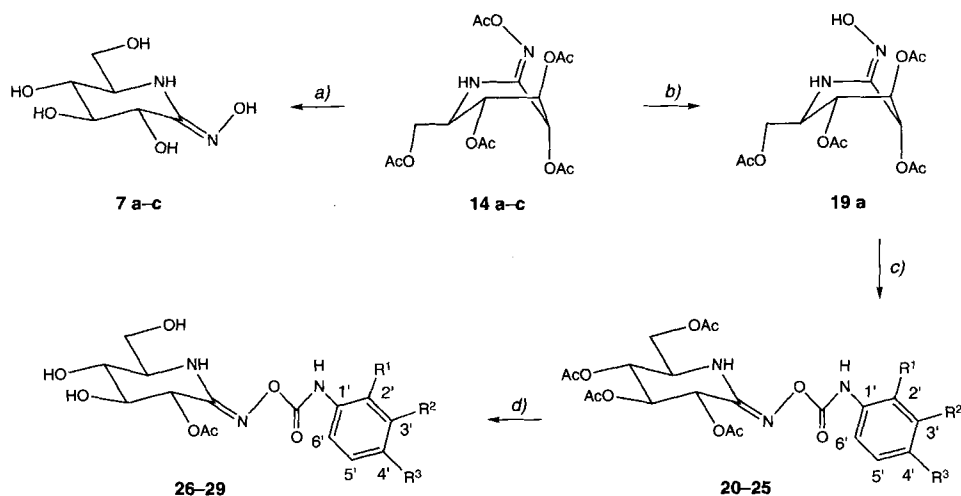


Fig. 2. ORTEP Representation of **7**Fig. 3. ORTEP Representation of **21**

ether **12a** with  $^{15}\text{NH}_2\text{OH}$ , followed by debenzylation, acetylation, and deacetylation, as described for **7a**.

Only the structures **7**, **13**, and **14** for the isotomeric, unprotected, and protected hydroximo-lactams are compatible with the  $^{15}\text{N},^1\text{H}$  couplings and the chemical-shift values in the  $^{15}\text{N}$ -NMR spectra (Table 3) [6]. The  $^{15}\text{N}$ -NMR spectra of the hydroximo-lactams **7b**, **13b**, and **14b**, labeled at the endocyclic position, show *doublets* and those of **7c**, **13c**, and **14c**, labeled at the exocyclic position, show *singlets*, hence, the C=N bond must be exocyclic, and the endocyclic N-atom bears the H substituent. This is also evident

Scheme 2



<b>20, 26</b> R <sup>1</sup> = R <sup>2</sup> = R <sup>3</sup> = H	<b>23, 29</b> R <sup>3</sup> = Cl, R <sup>1</sup> = R <sup>2</sup> = H
<b>21, 27</b> R <sup>1</sup> = Cl, R <sup>2</sup> = R <sup>3</sup> = H	<b>24</b> R <sup>1</sup> = R <sup>3</sup> = Cl, R <sup>2</sup> = H
<b>22, 28</b> R <sup>2</sup> = Cl, R <sup>1</sup> = R <sup>3</sup> = H	<b>25</b> R <sup>2</sup> = R <sup>3</sup> = Cl, R <sup>1</sup> = H

*a*) NaOMe, MeOH, 0°, 48 h; 88%. *b*) BnNH<sub>2</sub>, THF, 0°, 12 h; 64%. *c*) ArNCO, THF, Et<sub>3</sub>N, r.t., 15 min; 62–79% from **14a**.  
*d*) NaOMe, MeOH, 0°, 5–7 h; 48–88%.

from the chemical-shift values. The exocyclic <sup>15</sup>N resonates at a far lower field ( $\Delta\delta \approx 200$  ppm) than the endocyclic one, in keeping with its essentially trigonal hybridization. The exocyclic C=N bond is confirmed by the X-ray analysis of **7** (Fig. 2). Its bond length (1.294 Å) is typical for oximes. The N(5)–C(1) bond (1.360 Å) is slightly longer than the N(5)–C(1) bond in glucono-lactam (1.326 Å) [24], but shorter than the C–N bond in piperidines (1.47 Å). This indicates a hybridization of N(5) between sp<sup>3</sup> and sp<sup>2</sup> with a lower s-character than the N-atom in glucono-lactam and a weaker conjugative interaction with the oximino than with the C=O group, as predicted by the calculations. This is confirmed by the pyramidalization of N–C(5) in **7**, as specified by the distance (0.23 Å) of N–C(5) from the plane defined by H–N(5), C(1), and C(5). By comparison, this distance amounts to 0.01 Å in glucono-lactam and to 0.42 Å in (1'S\*,2S\*,4R\*)-2-( $\alpha$ -hydroxybenzyl)-4-phenylpiperidine [25]. In the solid state, **7** adopts a conformation between <sup>4</sup>C<sub>1</sub> and <sup>4</sup>H<sub>3</sub> (torsion angle C(2)–C(1)–N(5)–C(5): –29.6°), in good agreement with calculations of the model compound, whereas glucono-lactam is an almost perfect <sup>4</sup>H<sub>3</sub> (torsion angle C(2)–C(1)–N(5)–C(5): –4.8°) [24]. It is, therefore, not surprising, that **7** adopts a <sup>4</sup>C<sub>1</sub> conformation in aqueous solution (*cf.* Table 4). These findings show a limitation of empirical formulae which correlate hybridization and <sup>15</sup>N, <sup>1</sup>H coupling constants [26], and which predict sp<sup>2</sup>-hybridization for a <sup>15</sup>N, <sup>1</sup>H coupling constant of *ca.* 90 Hz (Table 3).

Table 3.  $^{15}\text{N}$ -NMR Chemical Shifts  $\delta$  [ppm], Multiplicities, and NH Coupling Constants  $J$  [Hz] of  $^{15}\text{N}$ -labeled Lactam Oximes

Compound	Solvent	Endocyclic $^{15}\text{N}$		Exocyclic $^{15}\text{N}$
		$\delta$	$J$	
<b>13b</b>	$\text{C}_6\text{D}_6$	-306.8 ( <i>d</i> )	91	
<b>13c</b>	$\text{C}_6\text{D}_6$			-101.7 ( <i>s</i> )
<b>14b</b>	$\text{C}_6\text{D}_6$	-308.2 ( <i>d</i> )	93	
<b>14c</b>	$\text{C}_6\text{D}_6$			-81.8 ( <i>s</i> )
<b>7b</b>	$\text{H}_2\text{O}/\text{D}_2\text{O}$ 9:1	-305.5 ( <i>d</i> )	65 <sup>a)</sup>	
<b>7b</b>	$\text{H}_2\text{O}/\text{D}_2\text{O}$ , 5 equiv. AcOH	-280.6 <sup>b)</sup>		
<b>7c</b>	$\text{H}_2\text{O}/\text{D}_2\text{O}$ 9:1			-125.9 ( <i>s</i> )
<b>7c</b>	$\text{D}_2\text{O}$ , 5 equiv. AcOH			-209.8 <sup>b)</sup>
<b>7c</b>	( $\text{D}_6$ )DMSO, 1 equiv. $\text{CF}_3\text{CO}_2\text{H}$			-215.6 <sup>b)</sup>

<sup>a)</sup> Onset of coalescence. <sup>b)</sup> Proton decoupled.

Addition of AcOH or  $\text{CF}_3\text{COOH}$  to the isotopomer **7b** results in a shift of the  $^{15}\text{N}$  signal to lower field ( $\Delta\delta = 25$  ppm); similarly, addition of AcOH to **7c** causes a shift of the  $^{15}\text{N}$  signal to higher field ( $\Delta\delta = 84$  ppm). These strong protonation induced shifts demonstrate the basic character of the hydroximo-lactam **7**, for which we found a  $pK_{\text{HA}}$  value of 4.7–4.8 (compare also [4]). The convergent nature of these shifts shows that protonation of **7** leads to a stronger conjugative interaction of the endocyclic N-atom with the hydroxyimino group, as predicted by the calculations. This should result in a planarization of the oxime function. Calculations predict a concomitant change of the ring conformation towards a half-chair; charge and conformation are thus not independent of each other (*cf.* [4]). Addition of 1 equiv. of  $\text{CF}_3\text{COOH}$  induces a downfield shift of the signals of H–C(2 and 5) of 0.31, of H–C(3 and 4) of 0.15–0.20, of H'–C(6) of 0.04, and of H–C(6) of *ca.* 0.1 ppm, and a strong overlap of the signals of H–C(3, 4, and 6), with simultaneous change of the coupling pattern. The spectrum (200 MHz) did not change sufficiently upon heating the sample to 65° to allow a conformational analysis of protonated **7a** in  $\text{D}_2\text{O}$ .

To evaluate the effect of an *O*-Ac group on the  $^{15}\text{N}$  chemical shift of the hydroximo-lactams, we compared the spectra of **13c** and **14c** with those of 4-(*tert*-butyl)cyclohexanone oxime ( $\text{CDCl}_3$ ,  $\delta = -53.3$  ppm) and its acetate ( $\text{CDCl}_3$ ,  $\delta = -38.4$  ppm). The chemical-shift difference in the spectra of these reference compounds ( $\Delta\delta = 14.9$  ppm) is similar to the  $\Delta\delta$  for **13c** and **14c** (19.9 ppm). To evaluate the solvent effect in the  $^{15}\text{N}$ -NMR spectra of **7c** and **13c**, we also recorded the  $^{15}\text{N}$ -NMR spectra of acetone oxime in  $\text{D}_2\text{O}$  ( $\delta = -53.3$  ppm) and in  $\text{C}_6\text{D}_6$  ( $\delta = -45.0$  ppm). For acetone oxime, the solvent induced chemical shift difference ( $\Delta\delta = 8.3$  ppm) is considerably smaller than for **7c** and **13c** ( $\Delta\delta = 24.2$  ppm).

Partial deprotection of **14a** with  $\text{PhCH}_2\text{NH}_2$  yielded **19a**, which was treated with the appropriate isocyanates and transformed into the *N*-arylcarbamates **20–25**. Deprotection of the tetraacetates **20–25** was not trivial, and accompanied by formation of a by-product in trace amounts for **20**, but in 5–50% for **21–25** (*(E)*-isomer?). This by-product could be

removed by crystallization only, and the monochlorinated phenyl carbamates **27–29** derived from **21–23** were thus purified, while the procedure failed for the dichlorophenyl derivatives. In agreement with the calculations for 5-pentanelactam oxime, the coupling constants in the  $^1\text{H-NMR}$  of the deprotected carbamates **26–29** (cf. Table 6) indicate that these compounds adopt a  $^4\text{C}_1$  conformation in solution, and, at least in the case of the acetylated 2-chlorophenyl carbamate **21**, also in the solid state (torsion angle of  $\text{C}(2)\text{--C}(1)\text{--N}(5)\text{--C}(5)$ :  $-34.3^\circ$ ). The X-ray analysis of **21** (Fig. 3) demonstrates again the exocyclic  $\text{C}=\text{N}$  bond, with bond lengths of 1.279 and 1.366 Å for  $\text{N}(1)\text{--C}(1)$  and  $\text{N}(5)\text{--C}(1)$ , respectively.

The IR spectra (KBr) of the tetraacetylated carbamates **20–25** show a single, quite strong NH band (sh.) at ca.  $3460\text{ cm}^{-1}$ . Bands due to the  $\text{C}=\text{N}$  bond are found between  $1650$  and  $1660\text{ cm}^{-1}$ , and those due to the Ac groups between  $1740$  and  $1755\text{ cm}^{-1}$ . The  $^1\text{H-NMR}$  spectra show NH resonances between  $8.35\text{--}8.45$  (PhNH) and the expected signals in the aromatic region. All signals in the  $^1\text{H}$ - and  $^{13}\text{C-NMR}$  spectra were assigned on the basis of a  $^1\text{H},^{13}\text{C-COSY}$  experiment, several  $^1\text{H-NMR}$  homo-decoupling experiments, and, with regard to the carbamoyl moiety, according to [27].

The  $^1\text{H-NMR}$  spectra of the deprotected carbamates **26–29** in ( $\text{D}_6$ )DMSO show the expected ArH signals, ArNH singlets between  $9.32$  and  $9.65$  ppm, the ring NH s at around  $6.11$  ppm (except for **27**, where it is recorded at  $6.38$  ppm), and the typical resonances of the ring H of the hydroximo-lactam moiety, three secondary OH and one primary OH group.

**3. Evaluation of D-Gluconhydroximo-1,5-lactam (7) and of the N-Arylcarbamates 26–29 as Inhibitors.** The hydroximo-lactam **7** and the *N*-arylcarbamates **26–29** – particularly the 2-chlorophenyl carbamate **27** – are strong competitive inhibitors (see Table 4), of  $\beta$ -glucosidases from almonds<sup>1)</sup> and from *Agrobacterium faecalis*. These inhibitors are stronger than D-nojirilactam (**32**) [28], D-gluconhydroximo-1,5-lactone (**1**) [29], and the D-gluconhydroximo-1,5-lactone-derived phenyl carbamate **3** [1] [29], presumably due to their weakly basic character. Protonation increases the polar character of the functional group involving the anomeric center and may lead to a stronger interaction with the hypothetical anionic group at the active site of the enzyme. The *N*-arylcarbamates are indeed among the tightest binding inhibitors yet found [30], and the differences between the isomeric chlorophenyl carbamates suggest a specific interaction at the active site, although it is not clear why the 2-chlorophenyl carbamate **27** is the most potent inhibitor.

Table 4. Inhibition Constants ( $K_i$ ) for Lactone and Lactam Derivatives against the  $\beta$ -Glucosidases from Sweet Almonds (*Emulsin*) at pH 6.8 and from *Agrobacterium faecalis* (*Abg*) at pH 7.0

Compound	<b>1</b>	<b>3</b>	<b>5</b>	<b>7</b>	<b>26</b>	<b>27</b>	<b>28</b>	<b>29</b>	<b>32</b>	
<i>Emulsin</i>	$K_i$ [ $\mu\text{mol}$ ]		43	74	16	13	8	12	21	125
<i>Abg</i>	$K_i$ [ $\mu\text{mol}$ ]	30	1.4		0.6	1.2	0.15	0.9	0.8	5.2

<sup>1)</sup> For **7**, a  $K_i$  value of  $13.8\ \mu\text{mol}$  at pH 5.6 has been reported [4].

We thank the *Swiss National Science Foundation* and *F. Hoffmann-La Roche AG*, Basle, for generous support, and *Dr. A. Linden*, University of Zurich, for the X-ray analyses.

### Experimental Part

*General.* DMSO was obtained from freshly opened bottles and stored over 4-Å molecular sieves, other solvents were distilled. Reactions were run under Ar. TLC: *Merck* silica gel 60  $F_{254}$  plates; detection by heating with  $I_2$  soln./20%  $H_2SO_4$  1:1 ( $I_2$  soln.: 10 g of  $I_2$ , 100 g of KI, 1000 ml  $H_2O$ ) or with mostain [31]. Flash chromatography (FC): silica gel (*Merck* 60; 0.040–0.063 mm). M.p.: uncorrected. Except where noted otherwise,  $^1H$ -NMR spectra were recorded at 300 MHz,  $^{13}C$ -NMR spectra at 50 MHz, and  $^{15}N$ -NMR spectra at 40.5 MHz with  $MeNO_2$  as external reference. Chemical shifts  $\delta$  in ppm and coupling constants  $J$  in Hz.

**2,3,4,6-Tetra-O-benzyl-D-( $^{15}N$ )gluconamide (30).** At  $-120^\circ$ , condensed  $^{15}NH_3$  (ca. 0.77 g, 39 mmol) was treated with a soln. of crude 2,3,4,6-tetra-O-benzyl-D-gluconolactone (41 g, 76 mmol) [18] in  $CH_2Cl_2$  (140 ml). The mixture was kept at  $-60^\circ$  for 6 h, warmed to  $15^\circ$  within 14 h, heated to  $40^\circ$  for 30 min, and evaporated. Excess lactone was separated by FC (hexane/AcOEt 1:1  $\rightarrow$  AcOEt), and treated with  $NH_3$  as described in [18]. The crude amide **30** (8.4 g) was dissolved in boiling  $Et_2O$ , and the soln. cooled first to r.t., and then to  $5^\circ$ . Filtration and drying gave 5.12 g (24%) of crystalline **30**. FC of the mother liquor gave 0.75 g (3%) of **30** as an oil.  $^1H$ -NMR (400 MHz,  $C_6D_6$ ): 6.37 (*dd*,  $J = 86$ , 3.4, NH); 6.59 (*dd*,  $J = 86$ , 3.6, NH).  $^{15}N$ -NMR ( $C_6D_6$ ):  $-279.3$  ( $t$ ,  $J = 90$ ). CI-MS ( $NH_3$ ): 559 (9); 558 (42); 557 (100,  $[M + H]^+$ ).

**2,3,4,6-Tetra-O-benzyl-D-( $^{15}N$ )glucono-1,5-lactam (11b)** [18].  $^{13}C$ -NMR (75 MHz,  $C_6D_6$ ): 54.51 (*dd*,  $J = 8.9$ , C(5)); 69.65 (*t*); 73.39 (*t*); 74.56 (*t*, C(3)); 77.38 (*d*); 79.26 (*dd*,  $J = 7.6$ , C(2)); 82.88 (*d*); 127.77–128.72 (several *d*); 138.44 (*s*); 138.85 (*s*); 139.01 (*s*); 139.14 (*s*); 171.33 (*d*,  $J = 13.2$ , C(1)).  $^{15}N$ -NMR ( $C_6D_6$ ):  $-264.8$  (*dt*,  $J = 89$ , 3.1). CI-MS ( $NH_3$ ): 542 (7), 541 (37), 540 (100,  $[M + H]^+$ ), 323 (6).

**(2R,3S,4S,5R)-3,4,5-Tris(benzyloxy)-2-[(benzyloxymethyl)-6-ethoxy-2,3,4,5-tetrahydropyridine (12a).** A soln. of **11a** (500 mg, 0.93 mmol) in  $CH_2Cl_2$  (19 ml) was treated with a 1M soln. of  $Et_3OBF_4$  in  $CH_2Cl_2$  (2.8 ml). The mixture was stirred for 20 h at r.t., treated with 1M  $Et_3OBF_4$  soln. (2.8 ml), stirred for 6 h, cooled to  $0^\circ$ , treated with  $Et_3N$  (14.6 ml; 104.7 mmol), and stirred for 1 h at r.t. Dilution with  $CH_2Cl_2$  (100 ml), washing with half-sat. aq.  $NaHCO_3$  soln., drying of the org. phase ( $Na_2SO_4$ ), filtration, evaporation, and FC (hexane/AcOEt 3:1) afforded **12a** (221 mg, 42%) and **11a** (108 mg, 22%). Solid.  $R_f$  (hexane/AcOEt 2:1) 0.70.  $[\alpha]_D^{25} = +103.6$  ( $c = 1.615$ ,  $CHCl_3$ ). IR ( $CHCl_3$ ): 3065w, 3030w, 3000m, 2990m, 2905m, 2890m, 1950w, 1875w, 1810w, 1675s, 1610w, 1590w, 1500m, 1485w, 1460m, 1390w, 1365m, 1315w, 1300m, 1270w, 1240w, 1190w, 1095s, 1070s (sh), 1030s, 915w, 830w, 700s.  $^1H$ -NMR (400 MHz,  $C_6D_6$ ): 1.12 (*t*,  $J = 7.0$ ,  $OCH_2CH_3$ ); 3.67 (*ddd*,  $J \approx 8.7$ , 4.5, 2.8; irradi. at 4.08; *dt*,  $J = 8.6$ , 2.8, H-C(2)); 3.81 (*dd*,  $J = 9.2$ , 2.7, CH-C(2)); 3.85 (*dd*,  $J = 9.2$ , 3.3, CH-C(2)); 3.91 (*t*,  $J = 8.9$ , H-C(3)); 3.99 (*dd*,  $J = 9.4$ , 7.4, H-C(4)); 4.08 (*dd*,  $J = 7.4$ , 1.5; irradi. at 3.67; *d*,  $J = 7.3$ , H-C(5)); 4.18 (*q*,  $J = 6.9$ ,  $OCH_2CH_3$ ); 4.38 (*d*,  $J = 12.2$ ), 4.45 (*d*,  $J = 12.2$ ,  $PhCH_2$ ); 4.62 (AB,  $J = 10.2$ ,  $PhCH_2$ ); 4.74 (*d*,  $J = 11.6$ ), 4.80 (*d*,  $J = 11.6$ ,  $PhCH_2$ ); 4.93 (*d*,  $J = 11.4$ ); 4.97 (*d*,  $J = 11.4$ ,  $PhCH_2$ ); 7.02–7.40 (*m*, 20 arom. H).  $^{13}C$ -NMR ( $CDCl_3$ ): 14.25 (*q*); 60.45 (*d*); 61.28 (*t*); 70.43 (*t*); 73.11 (*t*); 74.47 (*t*); 74.60 (*t*); 74.72 (*t*); 77.10 (*d*); 79.07 (*d*); 83.15 (*d*); 127.35–128.78 (several *d*); 137.97 (*s*); 138.34 (*s*); 138.44 (*s*); 138.55 (*s*); 161.11 (*s*). CI-MS ( $NH_3$ ): 568 (8), 567 (40), 566 (100,  $[M + H]^+$ ), 504 (8), 391 (15), 110 (8), 52 (14). Anal. calc. for  $C_{36}H_{39}NO_5$  (565.71): C 76.43, H 6.95, N 2.48; found: C 76.39, H 6.91, N 2.31.

**2,3,4,6-Tetra-O-benzyl-D-gluconohydroximo-1,5-lactam (13a).** a) *Via 12a.* The lactam **11a** (3.100 g, 5.77 mmol) was treated with a 1.0M soln. of  $Et_3O \cdot BF_4$  in  $CH_2Cl_2$  (11.9 ml), stirred at r.t. for 24 h, treated with 1.0M  $Et_3O \cdot BF_4$  soln. (2.96 ml), and stirred for further 4.5 h.  $Et_3N$  (4.13 ml, 29.6 mmol) was added in such a rate that the temp. was kept  $\leq 0^\circ$ . The mixture was warmed to r.t. within 1 h, diluted with  $CH_2Cl_2$  (100 ml), and washed with half-sat. aq.  $NaHCO_3$  soln. (20 ml). The org. phase was dried ( $Na_2SO_4$ ) and filtered, and the filtrate was evaporated. A soln. of the residue in dry MeOH (45 ml) was treated with 4-Å molecular sieves (ca. 2 g) and a soln. of  $NH_2OH$  [32]<sup>12</sup> (0.783 g, 23.7 mmol) in dry MeOH (15 ml). The mixture was stirred for 40 min. Filtration through *Celite*, evaporation, and FC (toluene/AcOEt 7:1) gave **13a** (1.50 g, 47%) and **11a** (0.98 g, 31%).

b) *From 15a/16a* (9:1 mixture of isomers). A soln. of **15a/16a** (7.25 g, 13.09 mmol) in dry MeOH (150 ml) was treated with  $NH_2OH \cdot HCl$  (1.128 g, 16.23 mmol) and  $NaHCO_3$  (1.364 g, 16.24 mmol), and kept under reflux for 2 h. Filtration, evaporation, and FC (hexane/AcOEt 2:1) gave **13a** (6.637 g, 92%) as a 10:1 ( $^1H$ -NMR) mixture in favor of the *gluco*-epimer. Anal. calc. for  $C_{34}H_{36}N_2O_5$  (552.67): C 73.89, H 6.57, N 5.07, found: C 74.09, H 6.68, N 5.09.

*Data of 13a:*  $R_f$  (hexane/AcOEt 2:1) 0.23.  $[\alpha]_D^{25} = +59.2$  ( $c = 0.75$ ,  $\text{CHCl}_3$ ). IR ( $\text{CCl}_4$ ): 3620w, 3430w, 3240w (br.), 3090w, 3070w, 3040m, 2920m, 2860m, 1670s, 1560w, 1540w, 1500m, 1460s, 1430w, 1390w, 1365m, 1320w, 1260w, 1210m, 1100s, 1070s (sh), 1030m, 965w (sh), 930w (sh), 910w, 730m, 700s.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ): 3.42 (dd,  $J = 9.9, 6.7$ , H-C(6)); 3.45 (dd,  $J = 5.5, 4.2$ , H-C(4)); 3.63 (dd,  $J = 9.9, 3.0$ , H-C(6)); 3.64–3.71 (m, H-C(5)); 3.84 (dd,  $J = 4.3, 2.7$ , H-C(3)); 3.97 (d,  $J = 2.5$ , H-C(2)); 4.27 (d,  $J = 11.5$ ), 4.44 (d,  $J = 11.5$ ,  $\text{PhCH}_2$ ); 4.33 (d,  $J = 11.7$ ), 4.53 (d,  $J = 11.7$ ,  $\text{PhCH}_2$ ); 4.38 (d,  $J = 12.1$ ), 4.67 (d,  $J = 12.1$ ,  $\text{PhCH}_2$ ); 4.44 (d, partially hidden), 4.47 (d,  $J = 10.6$ ,  $\text{PhCH}_2$ ); 5.46 (s, NH); 7.04–7.10 (m, 2 arom. H); 7.16–7.45 (m, 18 arom. H, OH).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ ): 51.39 (d); 69.32 (t); 70.66 (t); 71.77 (t); 72.37 (t); 73.09 (t); 74.12 (d); 80.50 (d); 82.20 (d); 127.64–128.36 (several d); 137.54 (s); 137.72 (s, 2 C); 137.89 (s); 149.79 (s). CI-MS ( $\text{NH}_3$ ): 555 (7), 554 (38), 553 (100,  $[\text{M} + \text{H}]^+$ ), 535 (9), 429 (15), 419 (10), 323 (8), 321 (5). Anal. calc. for  $\text{C}_{34}\text{H}_{36}\text{N}_2\text{O}_5$  (552.67): C 73.89, H 6.57, N 5.07; found: C 73.71, H 6.32, N 4.89.

*2,3,4,6-Tetra-O-benzyl-D-(5- $^{15}\text{N}$ )gluconhydroximo-1,5-lactam (13b).*  $^1\text{H-NMR}$  ( $\text{C}_6\text{D}_6$ ): 5.98 (d,  $J = 91$ , NH).  $^{13}\text{C-NMR}$  (75 MHz,  $\text{C}_6\text{D}_6$ ): 51.94 (dd,  $J = 9.9$  C(5)); 69.46 (t); 71.03 (t); 71.82 (t); 72.56 (t); 73.06 (t); 74.90 (d); 81.53 (d); 83.35 (d); 127.77–129.25 (several d); 138.44 (s); 138.51 (s); 138.58 (s); 138.93 (s); 150.10 (d,  $J = 13.3$ , C(1)). CI-MS ( $\text{NH}_3$ ): 556 (7), 555 (37), 554 (100,  $[\text{M} + \text{H}]^+$ ), 430 (10).

*2,3,4,6-Tetra-O-benzyl-D-glucon( $^{15}\text{N}$ )hydroximo-1,5-lactam (13c).*  $^{15}\text{NH}_4\text{OH} \cdot \text{HCl}$  (88 mg, 1.25 mmol) and  $\text{NaHCO}_3$  (104 mg, 1.24 mmol) were added to a soln. of **12a** (519 mg) in MeOH (20 ml). After completion of the reaction (TLC), the solvent was evaporated, and the residue purified by FC (hexane/AcOEt 1:1). Yield: 437 mg (86%).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ): identical to that of **13a**. CI-MS ( $\text{NH}_3$ ): 556 (8), 555 (37), 554 (100,  $[\text{M} + \text{H}]^+$ ), 432 (6), 430 (16), 324 (7).

*N-Acetoxy-2,3,4,6-tetra-O-benzyl-D-gluconhydroximo-1,5-lactam (31a).* A soln. of **13a** (100 mg, 0.18 mmol) in pyridine (1.2 ml) was treated with  $\text{Ac}_2\text{O}$  (0.1 ml, 1.06 mmol) and stirred for 30 min at r.t. TLC: completion of the reaction. Aq. workup, extraction with  $\text{CHCl}_3$ , and FC (hexane/AcOEt 3:1) gave **31a** (63.9 mg, 60%). Colorless oil.  $R_f$  (hexane/AcOEt 3:1) 0.28.  $[\alpha]_D^{25} = 41.1$  ( $c = 0.57$ , MeOH). IR ( $\text{CHCl}_3$ ): 3420m, 3000m, 2920w, 2860m, 1755s, 1645s, 1495m, 1450m, 1360m, 1240m, 1195m, 1090s, 1070s, 695s.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ): 2.10 (s, AcO); 3.39 (dd,  $J = 9.7, 7.0$ , H-C(6)); 3.43 (dd,  $J = 9.6, 3.2$ , H-C(4)); 3.64 (dd,  $J = 9.7, 3.2$ , H-C(6)); 3.70–3.77 (m, H-C(5)); 3.88 (dd,  $J = 3.3, 2.3$ , H-C(3)); 4.20 (d,  $J = 2.3$ , H-C(2)); 4.22 (d,  $J = 11.8$ ), 4.41 (d,  $J = 12.1$ ,  $\text{CH}_2\text{Ph}$ ); 4.30 (d,  $J = 11.7$ ), 4.56 (d,  $J = 11.7$ ,  $\text{PhCH}_2$ ); 4.38 (d,  $J = 12.0$ ), 4.45 (d,  $J = 12.1$ ,  $\text{PhCH}_2$ ); 4.51 (d,  $J = 12.0$ ), 4.68 (d,  $J = 12.0$ ,  $\text{PhCH}_2$ ).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ ): 19.74 (q); 51.13 (d); 68.92 (t); 70.89 (t); 71.69 (t); 72.00 (t); 73.05 (t); 73.67 (d); 80.29 (d); 80.96 (d); 127.71–128.49 (several d); 137.12 (s); 137.38 (s); 137.47 (s); 137.55 (s); 152.82 (s); 168.27 (s). CI-MS: 595 (5,  $[\text{M} + \text{H}]^+$ ), 432 (10), 431 (35), 429 (6), 366 (6), 365 (24), 337 (12), 325 (12), 324 (40), 323 (100), 321 (12). Anal. calc. for  $\text{C}_{36}\text{H}_{38}\text{N}_2\text{O}_6$  (594.70): C 72.70, H 6.44, N 4.71; found: C 72.67, H 6.69, N 4.48.

*2,3,4,6,N-Penta-O-acetyl-D-gluconhydroximo-1,5-lactam (14a) and 2,3,4,6,N-Penta-O-acetyl-D-mannonhydroximo-1,5-lactam (18a).* a) From **13a/17a** (10:1). A soln. of **13a/17a** (1.196 g, 2.16 mmol) in dry THF (13 ml) was added to a deep blue soln. of Na (0.50 g, 21.7 g-atom) in condensed  $\text{NH}_3$  (ca. 25 ml) at  $-60^\circ$  within 5 min. The cooling bath was removed, and the mixture was kept at reflux for 15 min, cooled to  $-60^\circ$ , and treated with  $\text{NH}_4\text{Cl}$  (1.2 g, 22 mmol). After evaporation, the residue was dissolved in MeOH, and filtered through *Celite*. The filtrate was evaporated, and the residue was dissolved in pyridine (10 ml), and treated with  $\text{Ac}_2\text{O}$  (3 ml) in the presence of a cat. amount of 4-(dimethylamino)pyridine. The mixture was taken to dryness, and the residue was dissolved in  $\text{CH}_2\text{Cl}_2$  (100 ml) and washed with sat. aq.  $\text{NaHCO}_3$  soln. Drying of the org. phase ( $\text{MgSO}_4$ ), evaporation, and FC (hexane/AcOEt 2:3) gave **14a** (405 mg, 47%), **14a/18a** (190 mg, 22%), and impure **18a** (94 mg, 11%) which, upon a second FC, afforded pure **18a** (23 mg, 3%).

b) **14a** from Pure **13a**. Similarly, pure **13a** (1.46 g, 2.64 mmol) was debenzylated and acetylated to yield **14a** (0.72 g, 68%).

*Data of 14a:* M.p. 100–101°.  $R_f$  (hexane/AcOEt 1:2) 0.28.  $[\alpha]_D^{25} = +89.7$  ( $c = 1.71$ ,  $\text{CHCl}_3$ ). IR ( $\text{CHCl}_3$ ): 3420w, 3380w, 3020m, 3000m, 1765s, 1660s, 1435m, 1370s, 1245s, 1210w, 1195s, 1045s, 1005m, 940m, 910m, 835w.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ): see Tables 5 and 6; AcO: 2.01 (s); 2.03 (s); 2.07 (s); 2.08 (s); 2.12 (s).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ ): see Table 7; AcO: 19.38 (q); 20.44 (q, 2 C); 20.52 (q, 2 C); 168.21 (s); 168.38 (s); 168.84 (s); 169.22 (s); 171.21 (s). CI-MS ( $\text{NH}_3$ ): 404 (17), 403 (100,  $[\text{M} + \text{H}]^+$ ), 345 (8). Anal. calc. for  $\text{C}_{16}\text{H}_{22}\text{N}_2\text{O}_{10}$  (402.36): C 47.76, H 5.51, N 6.96; found: C 47.68, H 5.37, N 7.11.

<sup>12)</sup> The preparation of  $\text{NH}_2\text{OH}$  *in situ* from  $\text{NH}_2\text{OH} \cdot \text{HCl}$  and  $\text{NaHCO}_3$  in MeOH or, alternatively, the use of *O*-(trimethylsilyl)hydroxylamine resulted in similar yields.







Table 7.  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ ) Chemical Shifts  $\delta$  [ppm] for Lactam Oximes and Lactam-Oxime Carbamates<sup>a)</sup>

	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)	$\text{O}_2\text{CN}$	C(1')	C(2')	C(3')	C(4')	C(5')	C(6')
<b>14a</b>	150.69	67.63	71.44	69.93	51.99	62.24							
<b>18a</b>	150.56	65.30	69.58	66.16	54.51	64.08							
<b>19a</b>	146.96	67.30	71.94	69.50	51.89	62.84							
<b>20</b>	149.03	67.35	71.72	69.85	51.85	62.34	151.84	136.89	119.51	129.02	124.19	129.02	119.51
<b>21</b>	149.44	66.87	71.27	68.99	52.59	62.50	151.38	133.95	122.95	127.66	124.26	120.24	128.95
<b>22</b>	149.30	67.33	71.71	69.86	51.90	62.32	151.56	138.18	129.98	134.68	119.45	117.44	124.17
<b>23</b>	149.28	67.28	71.66	69.79	51.90	62.29	151.71	135.58	128.95	120.67	129.12	120.67	128.95
<b>24</b>	149.60	66.95	71.36	69.10	52.61	62.54	151.31	132.85	128.85	128.72	123.52	121.00	127.90
<b>25</b>	149.42	67.31	71.74	69.89	51.89	62.29	151.49	136.56	130.49	132.80	127.36	121.02	118.67
<b>7<sup>b)</sup></b>	154.58	68.71	75.01	68.81	57.37	61.18							
<b>26<sup>b)</sup></b>	155.48	68.33	74.38	68.87	57.76	60.94	157.17	136.82	129.43	121.33	125.18	121.33	129.43
<b>27<sup>c)</sup></b>	152.21	69.67	75.21	71.16	56.63	61.21	155.49	134.35	134.35	127.79	122.39	124.23	129.29
<b>28<sup>c)</sup></b>	152.16	69.52	75.54	70.85	56.59	61.39	155.52	139.99	130.47	133.14	118.25	117.33	122.62
<b>29<sup>c)</sup></b>	152.35	69.61	75.63	70.94	56.76	61.53	155.49	137.47	128.64	120.62	126.66	120.62	128.64

a) Resonances for AcO; see *Exper. Part*. b) In  $\text{D}_2\text{O}$ . c) In  $(\text{D}_6)\text{DMSO}$ . d) At 150 MHz. e) Interpretation based upon a  $^1\text{H}$ ,  $^{13}\text{C}$ -COSY-NMR spectrum.

*Data of 18a*:  $R_f$  (hexane/AcOEt 1:2) 0.23. IR (CHCl<sub>3</sub>): 3400w, 3030w, 2990w, 1750s, 1700w (sh), 1650m (sh), 1640m, 1415w, 1360m, 1210m (br.), 1070m, 1045m, 1000w, 950w, 930w, 900w, 870w, 840w. <sup>1</sup>H-NMR: see *Tables 5 and 6*; AcO: 2.04 (s); 2.11 (s); 2.14 (s, 6H); 2.20 (s). <sup>13</sup>C-NMR: see *Table 7*; AcO: 19.60 (q); 20.44 (q); 20.59 (q, 3C); 168.55 (s); 168.85 (s); 169.40 (s); 169.52 (s); 170.56 (s). CI-MS (NH<sub>3</sub>): 420 (5, [M + NH<sub>4</sub>]<sup>+</sup>), 404 (17), 403 (100, [M + H]<sup>+</sup>), 345 (18).

*2,3,4,6-N-Pentaacetyl-D-glucon(5-<sup>15</sup>N)hydroximo-1,5-lactam (14b)*. <sup>1</sup>H-NMR (400 MHz, C<sub>6</sub>D<sub>6</sub>): 1.58 (s, AcO); 1.58 (s, AcO); 1.59 (s, AcO); 1.66 (s, AcO); 1.89 (s, AcO); 3.40–3.44 (m, H–C(5)); 3.83 (dt,  $J \approx 11.8, 5.2$ , H–C(6)); 3.93 (dt,  $J \approx 12.2, 4.4$ , H'–C(6)); 5.18 (dd,  $J \approx 9.4, 6.0$ , H–C(4)); 5.56 (t,  $J \approx 5.7$ , H–C(3)); 5.77 (d,  $J \approx 5.6$ , H–C(2)); 5.79 (dd,  $J = 9.3, 0.9$ , NH).

*2,3,4,6,N-Pentaacetyl-D-(<sup>15</sup>N)gluconhydroximo-1,5-lactam (14c)*. <sup>1</sup>H-NMR (CDCl<sub>3</sub>): identical to that of **14a**. CI-MS (NH<sub>3</sub>): 406 (4), 405 (18), 404 (100, [M + H]<sup>+</sup>).

*2,3,4,6-Tetra-O-benzyl-D-glucothionolactam (15a)*. A mixture of *2,3,4,6-tetra-O-benzyl-D-glucono-1,5-lactam (11a)*, 7.04 g, 13.09 mmol [18] and *Lawesson's reagent* (3.44 g, 8.51 mmol) [22] in dry C<sub>6</sub>H<sub>6</sub> (200 ml) was heated to reflux for 2 h. Evaporation and FC (toluene/AcOEt 20:1) gave **15a** (7.25 g, 99%) which was crystallized from hexane/AcOEt to give a 9:1 mixture **15a/16a** (<sup>1</sup>H-NMR) with **15a** as the major constituent. M.p. 85–86° (hexane/AcOEt; 1st fraction of epimeric mixture).  $R_f$  (toluene/CH<sub>2</sub>Cl<sub>2</sub> 1:1) 0.25. IR (KBr): 3145m, 3055w, 3020m, 2900m, 2860m, 1605w, 1545s, 1520w (sh), 1495m, 1460w (sh), 1455s, 1410m, 1400m, 1360m, 1345m, 1320m, 1280w, 1255w, 1230w, 1205m, 1165m, 1135s, 1160s (sh), 1095s, 1060s, 1025m, 990m, 935w, 905m, 855w, 820w. <sup>1</sup>H-NMR (CDCl<sub>3</sub>; *gluco-epimer*): 3.35 (dd,  $J = 9.8, 7.3$ , H–C(6)); 3.54 (dd,  $J = 9.4, 4.7$ , H–C(4)); 3.60 (dd,  $J = 9.8, 3.3$ , H'–C(6)); 3.85 (m, H–C(5)); 3.87 (t,  $J = 4.5$ , H–C(3)); 4.32 (d,  $J = 11.4$ , PhCH); 4.40–4.44 (m, 3 PhCH, H–C(2)); 4.55 (d,  $J = 11.5$ ), 4.64 (d,  $J = 11.5$ , PhCH<sub>2</sub>); 4.71 (d,  $J = 11.5$ ), 4.99 (d,  $J = 11.5$ , PhCH<sub>2</sub>); 7.10–7.20 (m, 2 arom. H); 7.21–7.36 (m, 16 arom. H); 7.42–7.37 (m, 2 arom. H); 8.16 (s, NH). <sup>13</sup>C-NMR (CDCl<sub>3</sub>; *gluco-epimer*): 55.91 (d); 68.24 (t); 72.44 (t); 72.51 (t); 72.69 (t); 73.32 (t); 78.32 (d); 81.26 (d); 82.44 (d); 127.67–128.60 (several d); 137.03 (s); 137.33 (s, 2 C); 137.44 (s); 200.29 (s). CI-MS (NH<sub>3</sub>; epimeric mixture): 556 (11), 555 (36), 554 (100, [M + H]<sup>+</sup>), 449 (10), 448 (38), 446 (18), 338 (37), 108 (14). Anal. calc. for C<sub>34</sub>H<sub>35</sub>NO<sub>4</sub>S (553.71; epimeric mixture): C 73.75, H 6.37, N 2.53, S 5.79; found: C 73.80, H 6.41, N 2.50, S 5.90.

*2,3,4,6-Tetra-O-acetyl-D-gluconhydroximo-1,5-lactam (19a)*. A soln. of **14a** (225 mg, 0.56 mmol) in dry THF (11.2 ml) was cooled to 0°, treated with distilled PhCH<sub>2</sub>NH<sub>2</sub> (64 µl, 0.59 mmol), and stirred for 12 h at 0°. After removal of THF at 0° and PhCH<sub>2</sub>NH<sub>2</sub> at r.t., FC (toluene/Et<sub>2</sub>O 1:4) gave **19a** (130 mg, 64%). Syrup.  $R_f$  (toluene/Et<sub>2</sub>O 1:4) 0.28.  $[\alpha]_D^{25} = +93.6$  ( $c = 1.045$ , CHCl<sub>3</sub>). IR (CHCl<sub>3</sub>): 3585w, 3490m, 3270m (br.), 3020m, 2980m, 2970m, 2910w, 2860w, 1740s, 1655s, 1630m (sh), 1470m, 1450m (sh), 1420m, 1360s, 1220s, 1030s, 970w, 930m, 915m, 890m. <sup>1</sup>H-NMR: see *Tables 5 and 6*; AcO: 2.04 (s); 2.07 (s); 2.10 (s, 6H). <sup>13</sup>C-NMR: see *Table 7*; AcO: 20.42 (q, 4C); 168.91 (s); 169.24 (s); 169.31 (s); 170.57 (s). CI-MS (NH<sub>3</sub>): 362 (15), 361 (100, [M + H]<sup>+</sup>), 243 (5), 225 (18), 130 (7). Anal. calc. for C<sub>14</sub>H<sub>20</sub>N<sub>2</sub>O<sub>9</sub> (360.32): C 46.67, H 5.59, N 7.77; found: C 46.84, H 5.50, N 7.78.

*General Procedure for the Preparation of the Carbamates 20–25*. The mixture resulting from selective deacetylation of **14a**, as described above, was treated with Et<sub>3</sub>N (3.0 equiv.) and the appropriate isocyanate (1.1 equiv.), and was stirred for 10 min. After removal of THF and PhCH<sub>2</sub>NH<sub>2</sub> at 40°, FC (hexane/AcOEt 3:1 → hexane/AcOEt 2:3) of the residue gave the pure carbamates.

*O-(2,3,4,6-Tetra-O-acetyl-1,5-dideoxy-1,5-imino-D-glucofuranosylidene)amino N-Phenylcarbamate (20)*. According to the *General Procedure*, **14a** (278 mg, 0.691 mmol) was converted to **20** (234 mg, 71%). Foam.  $R_f$  (hexane/AcOEt 1:2) 0.69.  $[\alpha]_D^{25} = +55.1$  ( $c = 1.75$ , CHCl<sub>3</sub>). IR (KBr): 3350s, 3120w, 3060w, 3020w, 2990w, 2980w, 2970w, 2950w, 1750s, 1660m, 1605m, 1550m (sh), 1525s (sh), 1515s, 1445s, 1370s, 1315w, 1300w, 1225s, 1080w (sh), 1045s, 995w, 940w, 905w, 865w, 835w, 760m, 690m. <sup>1</sup>H-NMR: see *Tables 5 and 6*; AcO: 2.08 (s, 6H); 2.12 (s); 2.16 (s). <sup>13</sup>C-NMR: see *Table 7*; AcO: 20.58 (q, 2C); 20.69 (q, 2C); 168.64 (s); 169.12 (s); 169.35 (s); 170.57 (s). CI-MS (NH<sub>3</sub>): 362 (16), 361 (100, [M – C<sub>7</sub>H<sub>5</sub>NO + H]<sup>+</sup>), 243 (4), 225 (15). Anal. calc. for C<sub>21</sub>H<sub>25</sub>N<sub>3</sub>O<sub>10</sub> (479.44): C 52.61, H 5.26, N 8.76; found: C 52.87, H 5.04, N 8.64.

*O-(2,3,4,6-Tetra-O-acetyl-1,5-dideoxy-1,5-imino-D-glucofuranosylidene)amino N-(2-Chlorophenyl)-carbamate (21)*. According to the *General Procedure*, **14a** (155 mg, 0.385 mmol) was converted to **21** (123 mg, 62%). White solid. M.p. 93–94° (hexane/Et<sub>2</sub>O/H<sub>2</sub>O).  $R_f$  (hexane/AcOEt 1:2) 0.78.  $[\alpha]_D^{25} = +41.8$  ( $c = 0.95$ , CHCl<sub>3</sub>). IR (KBr): 3620w, 3530w, 3480w, 3440m, 3000w, 2960w, 2920w, 2890w, 1750s (br.), 1655m, 1595m, 1580m, 1535s, 1465w, 1440m, 1380m (sh), 1370m, 1325w, 1300w, 1290w, 1230s, 1210s, 1190s (sh), 1130w, 1085w (sh), 1060m, 1030s, 1015m, 995m, 975w, 950w, 930w, 915m, 905w, 860w, 840w, 750m, 715w, 700w, 685w, 625w. <sup>1</sup>H-NMR: see *Tables 5 and 6*; AcO: 2.07 (s); 2.08 (s); 2.13 (s); 2.15 (s). <sup>13</sup>C-NMR: see *Table 7*; AcO: 20.45 (q, 2C); 20.49 (q); 20.59 (q); 168.65 (s); 169.14 (s); 169.27 (s); 170.46 (s). CI-MS (NH<sub>3</sub>): 404 (15), 403 (88, [M – C<sub>6</sub>H<sub>4</sub>Cl + H]<sup>+</sup>), 362 (14), 361 (100, [M – C<sub>7</sub>H<sub>4</sub>ClNO + H]<sup>+</sup>), 346 (8), 345 (53), 319 (35, [M –

$C_7H_4ClNO - C_7H_5O + 2 H^+$ ), 318 (37), 304 (7). Anal. calc. for  $C_{21}H_{24}ClN_3O_{10} \cdot 0.5 H_2O$  (522.90): C 48.24, H 4.82, N 8.04, Cl 6.78; found: C 48.25, H 4.82, N 8.04, Cl 7.09.

O-(2,3,4,6-Tetra-O-acetyl-1,5-dideoxy-1,5-imino-D-glucopyranosylidene)amino N-(3-Chlorophenyl)-carbamate (**22**). According to the *General Procedure*, **14a** (166 mg, 0.413 mmol) was converted to **22** (140 mg, 66%). White solid. M.p. 91–92° (hexane/Et<sub>2</sub>O).  $R_f$  (hexane/AcOEt 1:2) 0.72.  $[\alpha]_D^{25} = +53.3$  ( $c = 1.52$ , CHCl<sub>3</sub>). IR (KBr): 3480w, 3360m, 3115w, 3080w, 2980w, 2950m, 1755s, 1730s (sh), 1655m, 1600s, 1580m (sh), 1550m (sh), 1530s (sh), 1520s, 1490m, 1430m, 1410m, 1380m (sh), 1370m, 1335w, 1300m (sh), 1275m, 1245s, 1205s, 1170m (sh), 1120w, 1105m, 1085m (sh), 1045s, 1000m (sh), 950w, 925m, 900w, 880w, 865w, 780m, 750w, 725w, 685m. <sup>1</sup>H-NMR: see *Tables 5 and 6*; AcO: 2.12 (s, 6 H); 2.16 (s); 2.20 (s). <sup>13</sup>C-NMR: see *Table 7*; AcO: 20.55 (q, 2 C); 20.66 (q, 2 C); 168.62 (s); 169.07 (s); 169.32 (s); 170.59 (s). CI-MS (NH<sub>3</sub>): 362 (16), 361 (100,  $[M - C_7H_4ClNO + H]^+$ ), 301 (6), 243 (9), 225 (17). Anal. calc. for  $C_{21}H_{24}ClN_3O_{10}$  (513.89): C 49.08, H 4.71, N 8.18, Cl 6.90; found: C 48.87, H 4.95, N 8.42, Cl 7.05.

O-(2,3,4,6-Tetra-O-acetyl-1,5-dideoxy-1,5-imino-D-glucopyranosylidene)amino N-(4-Chlorophenyl)-carbamate (**23**). According to the *General Procedure*, **14a** (165 mg, 0.410 mmol) was converted to **23** (144 mg, 68%). Foam.  $R_f$  (hexane/AcOEt 1:2) 0.72.  $[\alpha]_D^{25} = +48.4$  ( $c = 0.875$ , CHCl<sub>3</sub>). IR (KBr): 3360s (sh), 3120w (sh), 2960w, 2940w, 1750s, 1660s, 1600s, 1590s, 1550m, 1520s (sh), 1505s, 1440m, 1410s, 1370s, 1310s, 1240s (sh), 1200s, 1120w, 1100s (sh), 1040s, 1010s, 940w, 910w, 870w, 830m, 750w, 680w (sh). <sup>1</sup>H-NMR: see *Tables 5 and 6*; AcO: 2.11 (s, 6 H); 2.15 (s); 2.19 (s). <sup>13</sup>C-NMR: see *Table 7*; AcO: 20.49 (q, 2 C); 20.61 (q, 2 C); 168.61 (s); 169.04 (s); 169.29 (s); 170.57 (s). Anal. calc. for  $C_{21}H_{24}ClN_3O_{10}$  (513.89): C 49.08, H 4.71, N 8.18, Cl 6.90; found: C 49.37, H 4.99, N 7.98, Cl 6.83.

O-(2,3,4,6-Tetra-O-acetyl-1,5-dideoxy-1,5-imino-D-glucopyranosylidene)amino N-(2,4-Dichlorophenyl)-carbamate (**24**). According to the *General Procedure*, **14a** (161 mg, 0.40 mmol) was converted to **24** (135 mg, 62%). White solid. M.p. 140.5–141.2° (hexane/Et<sub>2</sub>O).  $R_f$  (hexane/AcOEt 1:2) 0.76.  $[\alpha]_D^{25} = +44.0$  ( $c = 0.765$ , CHCl<sub>3</sub>). IR (KBr): 3460w, 3340s, 3120w, 3080w, 3020w (sh), 2980w, 2940m, 1770s, 1740s, 1660s, 1580s (sh), 1530s, 1515s, 1460m, 1430m, 1400s, 1380s, 1340m, 1310m, 1240s, 1220s, 1150m, 1130w, 1100m, 1060s, 1040s, 1020s, 1000m, 980m (sh), 950w, 920m, 910m, 870m, 850w, 820w, 800w, 750m (sh), 720w, 680w, 640m (sh), 610m. <sup>1</sup>H-NMR: see *Tables 5 and 6*; AcO: 2.07 (s); 2.08 (s); 2.13 (s); 2.14 (s). <sup>13</sup>C-NMR: see *Table 7*; AcO: 20.50 (q, 2 C); 20.55 (q); 20.64 (q); 168.62 (s); 169.16 (s); 169.30 (s); 170.52 (s). CI-MS (NH<sub>3</sub>): 362 (15), 361 (100,  $[M - C_7H_3Cl_2NO + H]^+$ ), 243 (11), 225 (13). Anal. calc. for  $C_{21}H_{23}Cl_2N_3O_{10}$  (548.33): C 46.00, H 4.23, N 7.66, Cl 12.93; found: C 46.21, H 4.23, N 7.72, Cl 12.76.

O-(2,3,4,6-Tetra-O-acetyl-1,5-dideoxy-1,5-imino-D-glucopyranosylidene)amino N-(3,4-Dichlorophenyl)-carbamate (**25**). According to the *General Procedure*, **14a** (170 mg, 0.423 mmol) was converted to **25** (184 mg, 79%). White solid. M.p. 106.5–107.5° (hexane/AcOEt).  $R_f$  (hexane/AcOEt 1:2) 0.71.  $[\alpha]_D^{25} = +47.0$  ( $c = 1.015$ , CHCl<sub>3</sub>). IR (KBr): 3460m (sh), 3360m, 2940w, 1750s, 1650m, 1600m, 1580m, 1520s, 1480m, 1430w, 1380s (sh), 1335w, 1300w, 1240s, 1210s, 1140w, 1050s, 920w, 890w, 870w, 820w, 750w, 690w. <sup>1</sup>H-NMR: see *Tables 5 and 6*; AcO: 2.09 (s, 6 H); 2.13 (s); 2.16 (s). <sup>13</sup>C-NMR: see *Table 7*; AcO: 20.55 (q, 2 C); 20.65 (q); 20.69 (q); 168.62 (s); 169.06 (s); 169.33 (s); 170.64 (s). CI-MS (NH<sub>3</sub>): 362 (20), 361 (100,  $[M - C_7H_3Cl_2NO + H]^+$ ), 243 (8), 225 (5). Anal. calc. for  $C_{21}H_{23}Cl_2N_3O_{10}$  (548.33): C 46.00, H 4.23, N 7.66, Cl 12.93; found: C 46.21, H 4.31, N 7.91, Cl 12.65.

*General Procedure for the Deacetylation of 14a–c and 20–23.* At 0°, 5 μl of a 0.22M soln. of NaOMe in MeOH were added to a soln. of the appropriate acetate in MeOH. After completion of the reaction (5–7 h), the soln. was neutralized (*Amberlite IR-120*), filtered, and the resin washed with MeOH (5–10 ml). Evaporation of the filtrate gave the crude polyols.

D-Gluconhydroximo-1,5-lactam (**7a**). According to the *General Procedure*, **14a** (246 mg, 0.611 mmol) was converted within 48 h at 0° to **7** (103 mg, 88%, after reversed-phase HPLC (MeCN/H<sub>2</sub>O 1:20)). Colorless crystals. M.p. 159.0–159.5° (EtOH).  $R_f$  (MeOH/AcOEt/H<sub>2</sub>O 7:2:1) 0.13.  $[\alpha]_D^{25} = 67.5$  ( $c = 0.42$ , MeOH). IR (KBr): 3400s (br.), 2960w, 2900w (br.), 1650s, 1560w, 1540w, 1320m, 1100s (sh), 1040s (sh), 940w, 870w. <sup>1</sup>H-NMR: see *Tables 5 and 6*. <sup>13</sup>C-NMR: see *Table 7*. ESI-MS: 225 (40,  $[M + H + MeOH]^+$ ), 193 (100,  $[M + H]^+$ ), 64 (18), 23 (18). Anal. calc. for  $C_6H_{12}N_2O_5$  (192.17): C 37.50, H 6.29, N 14.58; found: C 37.76, H 6.05, N 14.29.

D-(5-<sup>15</sup>N)Gluconhydroximo-1,5-lactam (**7b**). <sup>1</sup>H-NMR (400 MHz, H<sub>2</sub>O/D<sub>2</sub>O 9:1): 3.22–3.33 (m, H-C(5)); 3.59 (t,  $J = 9.0$ , H-C(4)); 3.65 (t,  $J = 9.4$ , H-C(3)); 3.70 (dt,  $J = 11.9, 4.6$ , H-C(6)); 3.84 (dt,  $J = 12.0, 3.0$ , H-C(6)); 4.15 (d,  $J = 9.0$ , H-(2)). CI-MS (NH<sub>3</sub>): 195 (7), 194 (100,  $[M + H]^+$ ), 178 (7), 142 (14).

D-Glucon(<sup>15</sup>N)hydroximo-1,5-lactam (**7c**). <sup>1</sup>H-NMR (D<sub>2</sub>O): identical to that of **7**. <sup>13</sup>C-NMR (D<sub>2</sub>O): 156.92 (d,  $J = 4.9$ , C(1)). CI-MS (NH<sub>3</sub>): 211 (11,  $[M + NH_4]^+$ ), 196 (6), 195 (36), 194 (100,  $[M + H]^+$ ), 193 (8), 178 (15), 176 (7).

O-(1,5-Dideoxy-1,5-imino-D-glucopyranosylidene)amino N-Phenylcarbamate (**26**). According to the *General Procedure*, **20** (157 mg, 0.327 mmol) was converted to **26** (102 mg). Reversed-phase HPLC (MeCN/H<sub>2</sub>O 1:2) and lyophilization afforded **26** (90 mg, 88%).  $R_f$  (MeOH/AcOEt/H<sub>2</sub>O 7:2:1) 0.67.  $[\alpha]_D^{25} = +12.7$  ( $c = 0.66$ , MeOH). IR (KBr): 3480s (br.), 1720s, 1660s, 1605s, 1555s, 1500w, 1450m, 1405m, 1320m, 1400w (sh), 1225m, 1140w, 1110m, 1085m, 1045m, 1025m, 980m, 910w, 880w, 845w, 800w, 755m, 690w. <sup>1</sup>H-NMR (400 MHz, (D<sub>6</sub>)DMSO): 3.18 (m, H-C(5)); 3.24 (m, H-C(4)); 3.37 (dt,  $J = 11.5, 4.7$ , H-C(6)); 3.53 (m, H-C(3)); 3.76 (ddd,  $J = 11.5, 5.7, 2.7$ , H-C(6)); 3.95 (dd,  $J = 6.1, 3.9$ , H-C(2)); 4.91 (t,  $J = 5.7$ , HO-C(6)); 5.16 (d,  $J = 5.4$ ), 5.23 (d,  $J = 4.3$ , HO-C(3), HO-C(4)); 5.52 (d,  $J = 3.9$ , HO-C(2)); 6.11 (s, H-N(1)); 7.04 (t,  $J = 7.6$ , H-C(4')); 7.31 (t,  $J = 7.6$ , H-C(3'), H-C(5')); 7.50 (d,  $J = 7.7$ , H-C(2'), H-C(6')); 9.41 (s, ArNH). <sup>13</sup>C-NMR: see Table 7. ESI-MS: 386 (15, [M + K]<sup>+</sup>), 350 (10, [M + K]<sup>+</sup>), 334 (100, [M + Na]<sup>+</sup>), 312 (20, [M + H]<sup>+</sup>), 250 (18). Anal. calc. for C<sub>13</sub>H<sub>17</sub>N<sub>3</sub>O<sub>6</sub> (311.29): C 50.16, H 5.50, N 13.50; found: C 50.36, H 5.66, N 13.30.

O-(1,5-Dideoxy-1,5-imino-D-glucopyranosylidene)amino N-(2-Chlorophenyl)carbamate (**27**). According to the *General Procedure*, **21** (0.126 g, 0.245 mmol) was converted to **27** (91 mg). Crystallization (MeOH/H<sub>2</sub>O) afforded 41 mg (48%). White solid. M.p. 160–161°.  $R_f$  (MeOH/AcOEt/H<sub>2</sub>O 7:2:1) 0.67.  $[\alpha]_D^{25} = +9.9$  ( $c = 0.42$ , MeOH). IR (KBr): 3440s (br.), 2950w, 2930w, 2880w, 1780w, 1760m, 1725s, 1640s, 1590s, 1580m (sh), 1525s, 1465w, 1440s, 1410w (sh), 1370w, 1350w, 1325w, 1305m, 1240w, 1200s, 1180m (sh), 1130m, 1110m, 1060s, 1020s, 990w, 950w, 900w, 880w, 850w, 830w, 800w, 740s, 710w, 690w, 655w. <sup>1</sup>H-NMR (600 MHz, (D<sub>6</sub>)DMSO): 3.12–3.23 (m, H-C(5)); 3.27–ca. 3.30 (m, superimposed by DMSO; addn. of D<sub>2</sub>O: dd,  $J = 9.0, 6.3$ , H-C(4)); 3.41 (dt,  $J = 11.3, 6.0$ , H-C(6)); 3.57 (dd,  $J = 10.2, 5.6$ ; irradi. at 3.93 ppm: t,  $J = 5.0$ ; addn. of D<sub>2</sub>O: t,  $J = 6.2$ , H-C(3)); 3.76 (ddd,  $J = 11.2, 5.7, 2.9$ , H-C(6)); 3.93 (t,  $J = 5.1$ , H-C(2)); 4.90 (t,  $J = 5.9$ , HO-C(6)); 5.19 (d,  $J = 5.5$ ), 5.23 (d,  $J = 4.3$ , HO-C(3), HO-C(4)); 5.62 (d,  $J = 4.6$ ; irradi. at 3.93 ppm: s, HO-C(2)), 6.38 (s, H-N(5)); 7.15 (dt,  $J = 7.8, 1.3$ ; irradi. at 7.94: t,  $J = 7.8$ , H-C(4')); 7.35 (dt,  $J = 7.3, 0.9$ ; irradi. at 7.94: dd,  $J = 7.3, 0.9$ , H-C(5')); 7.51 (dd,  $J = 8.0, 1.1$ ; H-C(3')); 7.94 (dd,  $J = 7.9, 1.1$ , H-C(2')); 7.94 (s, ArNH). <sup>13</sup>C-NMR: see Table 7. ESI-MS: 386 (5, [M + K]<sup>+</sup>), 384 (15, [M + K]<sup>+</sup>), 370 (37, [M + Na]<sup>+</sup>), 368 (100, [M + Na]<sup>+</sup>), 348 (5, [M + H]<sup>+</sup>), 346 (15, [M + H]<sup>+</sup>). Anal. calc. for C<sub>13</sub>H<sub>16</sub>ClN<sub>3</sub>O<sub>6</sub> (345.74): C 45.16, H 4.66, N 12.15, Cl 10.25; found: C 45.29, H 4.61, N 11.98, Cl 10.43.

O-(1,5-Dideoxy-1,5-imino-D-glucopyranosylidene)amino N-(3-Chlorophenyl)carbamate (**28**). According to the *General Procedure*, **22** (142 mg, 0.276 mmol) was converted to **28** (86 mg). Crystallization (MeOH/H<sub>2</sub>O) afforded 50 mg (53%). White solid. M.p. 123–124°.  $R_f$  (MeOH/AcOEt/H<sub>2</sub>O 7:2:1) 0.67.  $[\alpha]_D^{25} = +11.8$  ( $c = 0.68$ , MeOH). IR (KBr): 3340s (br.), 3100s (sh), 2900w, 1765m, 1730m, 1690s, 1655s, 1635s, 1490m, 1450w, 1430s, 1310m, 1280s, 1210s, 1170w, 1150m, 1105m, 1080m, 1020s, 1000w (sh), 980w, 930w, 900w, 880m, 780m, 755m, 710m, 685w, 655w. <sup>1</sup>H-NMR: see Tables 5 and 6. <sup>13</sup>C-NMR: see Table 7. ESI-MS: 384 (6, [M + K]<sup>+</sup>), 370 (37, [M + Na]<sup>+</sup>), 368 (100, [M + Na]<sup>+</sup>). Anal. calc. for C<sub>13</sub>H<sub>16</sub>ClN<sub>3</sub>O<sub>6</sub> (345.74): C 45.16, H 4.66, N 12.15, Cl 10.25; found: C 44.91, H 4.73, N 12.42, Cl 10.07.

O-(1,5-Dideoxy-1,5-imino-D-glucopyranosylidene)amino N-(4-Chlorophenyl)carbamate (**29**): According to the *General Procedure*, **23** (23.9 mg, 0.047 mmol) was converted to **29** (14.2 mg). Crystallization (toluene/MeOH) afforded 13.1 mg (82%). White solid. M.p. 108–110°.  $R_f$  (MeOH/AcOEt/H<sub>2</sub>O 7:2:1) 0.67.  $[\alpha]_D^{25} = +8.7$  ( $c = 0.80$ , MeOH). IR (KBr): 3420s (br.), 2940w, 2920w, 2880w, 2860w, 1730s, 1640s, 1600m, 1540m, 1530m, 1490m, 1400m, 1310m, 1290w, 1210s, 1140w, 1090m, 1025m, 1010m, 970w, 940w, 910w, 880w, 825m, 790w, 750w, 660w, 625w. <sup>1</sup>H-NMR: see Tables 5 and 6. <sup>13</sup>C-NMR: see Table 7. ESI-MS: 386 (10, [M + K]<sup>+</sup>), 384 (30, [M + K]<sup>+</sup>), 370 (35, [M + Na]<sup>+</sup>), 368 (100, [M + Na]<sup>+</sup>), 348 (3, [M + H]<sup>+</sup>), 346 (9, [M + H]<sup>+</sup>). Anal. calc. for C<sub>13</sub>H<sub>16</sub>ClN<sub>3</sub>O<sub>6</sub> (345.74): C 45.16, H 4.66, N 12.15, Cl 10.25; found: C 45.34, H 4.43, N 12.02, Cl 10.53.

*Enzyme-Inhibition Studies.* a) *Inhibition of Sweet Almond β-Glucosidase.* Inhibition constants ( $K_i$ ) of compounds listed in Table 4 were determined at 37° using a 0.08M KH<sub>2</sub>PO<sub>4</sub>/K<sub>2</sub>HPO<sub>4</sub> buffer (pH 6.8), and 4-nitrophenyl β-D-glucopyranoside (*Fluka*) as substrate. Measurements were started by addition of sweet almond β-glucosidase (*Emulsin, Fluka*). Enzyme activity was ca. 0.04 U/ml. The increase of absorption per min at 400 nm was taken as velocity for the hydrolysis of the substrate. This increase was linear during all measurements (1–3 min).  $K_M$  values were determined by means of *Lineweaver-Burk* plots [33]. They varied between 3.0 and 3.8 mM. The following substrate concentrations were applied: 19.91, 7.47, 4.15, 2.49, 1.66, and 1.16 mM.  $K_i$  values were determined by taking the slopes from the *Lineweaver-Burk* plots and plotting them against four to six inhibitor concentrations. After fitting the data to a straight line, the negative [I]-intercept of this plot gave the appropriate  $K_i$ .

b) *Inhibition of Agrobacterium faecalis β-Glucosidase.* *Agrobacter β-glucosidase (Abg)* was purified as described previously [34]. Buffer chemicals and substrates were obtained from *Sigma Chemical Company* or *BDH*. Enzyme assays were performed as described in a except that a buffer containing 50 mM Na<sub>2</sub>HPO<sub>4</sub> and 0.1% BSA (pH 7.0) was employed for all assays. Under these conditions the  $k_{cat}$  and  $K_M$  values of *Agrobacter β-*

glucosidase for 4-nitrophenyl  $\beta$ -D-glucopyranoside are 169 s<sup>-1</sup> and 78  $\mu$ M, respectively. Estimates of  $K_i$  values were obtained by measuring rates in a series of cells at a fixed substrate concentration (0.1 mM) in the presence of a range of inhibitor concentrations (6–10 concentrations) which encompassed the  $K_i$  value ultimately determined, generally from  $0.3 \times K_i$  to  $3 \times K_i$ . The observed rates were plotted in the form of a Dixon plot [35] and the  $K_i$  value determined from the intercept of this line with the horizontal line drawn through  $1/V_{\max}$ . Full  $K_i$  determinations were performed by measurement of rates at a series of substrate concentrations (typically 7 concentrations) which bracket the  $K_M$  value (generally  $0.15 \times K_M$  to  $7 \times K_M$ ) in the presence of a range of inhibitor concentrations (typically 5 concentrations) which bracket the  $K_i$  value ultimately determined. Data were analyzed by non-linear regression using the program *GraFit* [36].

## REFERENCES

- [1] D. Beer, A. Vasella, *Helv. Chim. Acta* **1986**, *69*, 267.
- [2] M. Horsch, L. Hoesch, A. Vasella, D. M. Rast, *Eur. J. Biochem.* **1991**, *197*, 815.
- [3] D. R. Wolk, A. Vasella, F. Schweikart, M. G. Peter, *Helv. Chim. Acta* **1992**, *75*, 323.
- [4] B. Ganem, G. Papandreou, *J. Am. Chem. Soc.* **1991**, *113*, 8984.
- [5] P. Ermert, A. Vasella, M. Weber, K. Rupitz, S. G. Withers, *Carbohydr. Res.*, accepted.
- [6] B. Clement, T. Kämpchen, *Chem. Ber.* **1985**, *118*, 3481.
- [7] M. J. S. Dewar, E. G. Zoebisch, E. F. Healy, J. J. P. Stewart, *J. Am. Chem. Soc.* **1985**, *107*, 3902.
- [8] W. J. Hehre, R. Ditchfield, J. A. Pople, *J. Chem. Phys.* **1972**, *56*, 2257.
- [9] P. C. Hariharan, J. A. Pople, *Theoret. Chim. Acta* **1973**, *28*, 213.
- [10] M. Häser, R. Ahlrichs, *J. Comp. Chem.* **1989**, *10*, 104.
- [11] G. La Manna, M. Cignitti, C. E. Notaro, *J. Mol. Struct. (THEOCHEM)* **1988**, *166*, 439.
- [12] G. A. Jeffrey, J. R. Ruble, R. K. McMullan, D. J. DeFrees, J. A. Pople, *Acta Cryst.* **1981**, *B37*, 1381.
- [13] M. T. Nguyen, T.-K. Ha, *J. Mol. Struct. (THEOCHEM)* **1982**, *88*, 127.
- [14] P. D. Adeney, W. J. Bouma, L. Radom, W. R. Rodwell, *J. Am. Chem. Soc.* **1980**, *102*, 4069.
- [15] M. K. Tong, G. Papandreou, B. Ganem, *J. Am. Chem. Soc.* **1990**, *112*, 6137.
- [16] S. Inouye, T. Tsuruoka, T. Ito, T. Niida, *Tetrahedron* **1968**, *23*, 2125.
- [17] G. W. J. Fleet, N. M. Carpenter, S. Petursson, N. G. Ramsden, *Tetrahedron Lett.* **1990**, *31*, 409.
- [18] R. Hoos, A. B. Naughton, A. Vasella, *Helv. Chim. Acta* **1993**, *76*, 1802.
- [19] *Jpn. Kokai Tokkyo Koho*, 80,105,666 to Nippon Shinyaku Co. [CA: **94** (1981) 103174e].
- [20] H. S. Overkleeft, J. v. Wiltenburg, U. K. Pandit, *Tetrahedron Lett.* **1993**, *34*, 2527.
- [21] C. M. McCloskey, *Adv. Carbohydr. Chem.* **1957**, *12*, 137.
- [22] S. Scheibye, B. S. Pedersen, S.-O. Lawesson, *Bull. Soc. Chim. Belg.* **1978**, *87*, 229.
- [23] B. M. Aebischer, *Dissertation Nr. 854*, Universität Freiburg, 1983.
- [24] H. Ogura, K. Furuhashi, H. Takayanagi, N. Tsuzuno, Y. Iitaka, *Bull. Chem. Soc. Jpn.*, **1984**, *57*, 2687.
- [25] P. Beak, W. K. Lee, *J. Org. Chem.* **1990**, *55*, 2578.
- [26] G. Binsch, J. B. Lambert, B. W. Roberts, J. D. Roberts, *J. Am. Chem. Soc.* **1964**, *86*, 5564.
- [27] E. P. Trub, E. N. Boitsov, B. M. Tsigin, *Zh. Org. Khim.* **1983**, *19*, 87.
- [28] M. P. Dale, H. E. Ensley, K. Kern, K. A. R. Sastry, L. D. Byers, *Biochemistry* **1985**, *24*, 3530.
- [29] S. G. Withers, K. Rupitz, D. Trimbur, R. A. J. Warren, *Biochemistry* **1992**, *31*, 9979.
- [30] G. Legler, *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 319.
- [31] P. Ermert, A. Vasella, *Helv. Chim. Acta* **1991**, *74*, 2043.
- [32] C. D. Hurd, *Inorg. Synth.* **1939**, *1*, 87.
- [33] H. Lineweaver, D. Burk, *J. Am. Chem. Soc.* **1934**, *56*, 658.
- [34] J. B. Kempton, S. G. Withers, *Biochem.* **1992**, *31*, 9961.
- [35] M. Dixon, *Biochem.* **1953**, *55*, 170.
- [36] R. J. Leatherbarrow, 'GraFit', Erithacus Software, Staines.
- [37] M. J. S. Dewar, K. M. Dieter, *J. Am. Chem. Soc.* **1986**, *108*, 8075.
- [38] W. J. Hehre, L. Radom, P. v. R. Schleyer, J. A. Pople, 'Ab initio molecular orbital theory', Wiley-Interscience, 1986.