# New Diabetogenic Streptozocin Analogue, 3-O-Methyl-2-{[(methylnitrosoamino) carbonyl]amino}-D-glucopyranose

## Evidence for a Glucose Recognition Site on Pancreatic B-Cells

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**SUMMARY**

**The nonmetabolizable glucose analogue 3-O-methyl-glucose is known to protect pancreatic B-cells against streptozocin (STZ) when injected with or just before STZ. If 3-O-methyl-glucose and the sugar moiety of STZ compete for a glucose recognition site on B-cells, it seemed likely that 3-O-methyl-2-deoxy-2-{[(methylnitrosoamino)carbonyl]amino}-D-glucopyranose, an analogue of STZ with a 3-O-methyl-glucosyl residue, would cause experimental diabetes. This possibility was tested by synthesis of this analogue (α-anomer) and comparison of its diabetogenic activity in Wistar rats with that of STZ. Results showed that the compound was diabetogenic and as potent as STZ. This new analogue is the first of the various STZ derivatives reported to show diabetogenic activity. Its activity supports the idea that 3-O-methyl-glucose and STZ bind competitively with a glucose recognition site on pancreatic B-cells. DIABETES 1986; 35:74–77.**

Streptozocin (STZ) is widely used for experimental induction of diabetes. The mechanism of its diabetogenic activity has been explained by supposing that DNA methylation is closely related to poly(ADP-ribose)synthetase in pancreatic B-cells.[1,2] However, the role of the carbohydrate moiety of STZ in the induction of diabetes has not been clarified. Since STZ showed antibacterial and antitumor activities,[3] many STZ derivatives have been synthesized, but none of them have been found to be diabetogenic.

The nonmetabolizable glucose analogues 3-O-methyl-glucose (3-OMe-G) and 2-deoxyglucose (2-DG) protect pancreatic B-cells from the toxicity of STZ when injected with or just before STZ.[4] We attempted to find a new diabetogenic derivative of STZ by examining the protective effect of glucose analogues and the diabetogenic activities of some STZ derivatives. This article reports these studies.

### MATERIALS AND METHODS

Male, 5-wk-old Wistar rats were used. STZ and its derivatives and/or glucose analogues as solutions in citrate buffer (pH 4.5) at the indicated doses were injected into the femoral vein under light ether anesthesia. Unless otherwise stated,

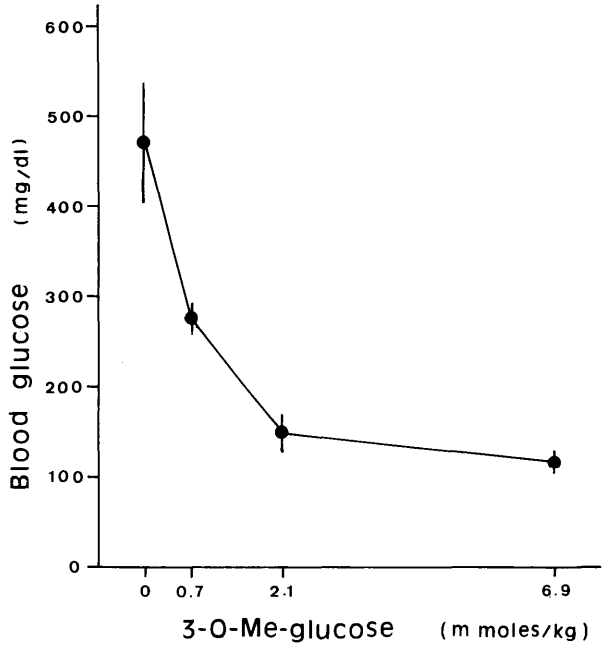


**FIGURE 1. Protective effect of 3-OMe-glucose against STZ (60 mg/kg)-induced hyperglycemia. The blood glucose values were determined at day 7 after injection. Six rats were used for each dose.**

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Received for publication 15 May 1985 and in revised form 30 July 1985.

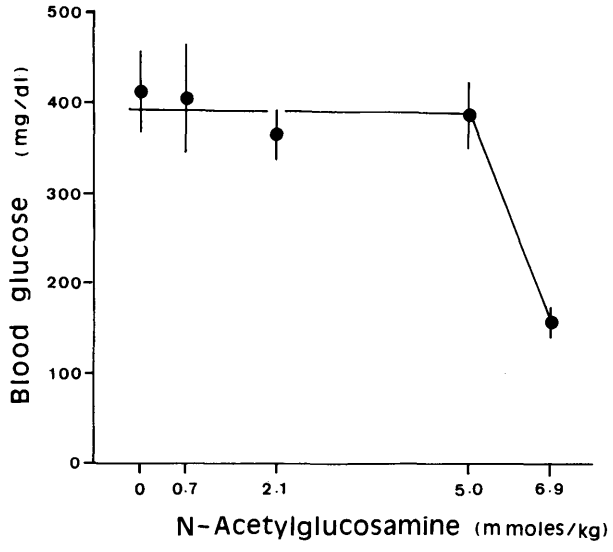
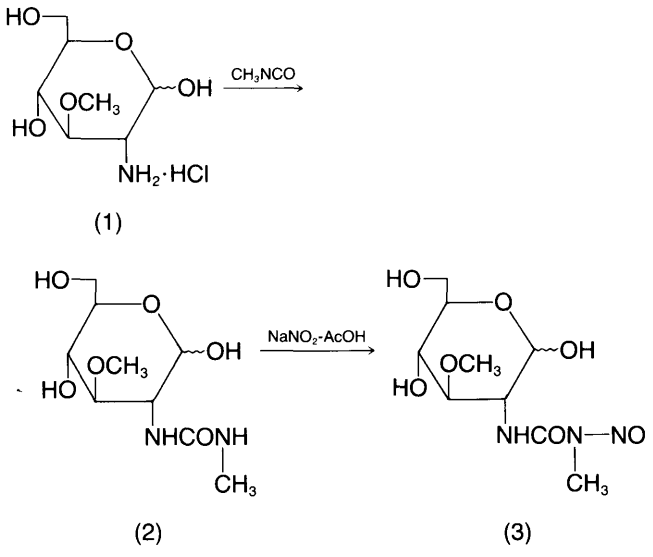



**FIGURE 2. Effect of N-acetylglucosamine on STZ (60 mg/kg)-induced hyperglycemia. The experimental conditions were the same as described in the legend to Figure 1.**

50-μl samples of blood were obtained from the tail vein every day from 2 to 5 days after the injection. Animals were fasted for 24 h on day 5 and then allowed access to food ad libitum. Blood samples from fasted (day 6) and fed (day 7) rats were also collected. The glucose concentration in blood samples was determined as described elsewhere[5] as an index of the diabetic state. STZ was purchased from Sigma, St. Louis, Missouri. α- and β-OMe-STZ (I and II) and $C_1$-β-methylnitrosourea-STZ (III) were synthesized. 3-OMe-G, 2-DG, and N-acetylglucosamine were purchased from Wako Pure Chemical Industries, Osaka, Japan. 3-O-methyl-α-methylglucoside (α-OMe-3-OMe-G) and 2-deoxy-α-methylglucoside (α-OMe-2-DG) were synthesized using Amberlite IR-120 ($H^+$ form) as a catalyst.[6]

A new compound, 3-O-methyl-2-deoxy-2-{[(methylnitrosoamino)carbonyl]amino}-D-glucopyranose (IV) (abbreviated as 3-OMe-STZ) was prepared from 3-O-methyl-glucosamine hydrochloride[7] applying the two-step process for STZ synthesis[8] according to the following scheme:




For example, to a solution of 2.30 g of 3-O-methylglucosamine hydrochloride (1) in 10 ml of water, 0.07 g of anhydrous $K_2CO_3$ and then 0.68 g of $CH_3NCO$ were added at 5°C under stirring. After 2 h, the reaction mixture was lyophilized and the residue was chromatographed on silica gel (solvent: ethylacetate/benzene/methanol, 5/2/2) to give 0.30 g of the α-anomer of 3-O-methyl-2-(3-methylureido)-2-deoxy-glucopyranose (2) and 1.50 g of the corresponding isomeric mixtures. A portion of the α-anomer (2) was subjected to structural analysis. The α-anomer of (2): amorphous powders, m.p. 148–149°C (dec.), $[\alpha]_D^{20}$ + 76.2° (c = 0.3, methanol). IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3400 (shoulder), 3340, 3320, 1630, 1590, 1040. NMR ($d_6$-DMSO) σ: 2.55 (3H, d, J = 5 Hz, $NCH_3$), 3.38 (3H, s, $OCH_3$), 4.37 (1H, t, J = 6 Hz, OH), 4.84 (1H, d-d, H-1; 4.87, d, J = 3.5 Hz after addition of $D_2O$), 4.97 (1H, d, J = 6 Hz, OH), 5.80 (2H, m, NH), 6.49 (1H, d, J = 5 Hz, OH).

The urea derivative (2, isomeric mixtures, 1.70 g) was dissolved in 15 ml of aqueous $CH_3COOH$ (15%), and 0.94 g of $NaNO_2$ was added portionwise at 5°C over a period of 30 min under stirring. After 30 min, 3 ml of 10% HCl solution

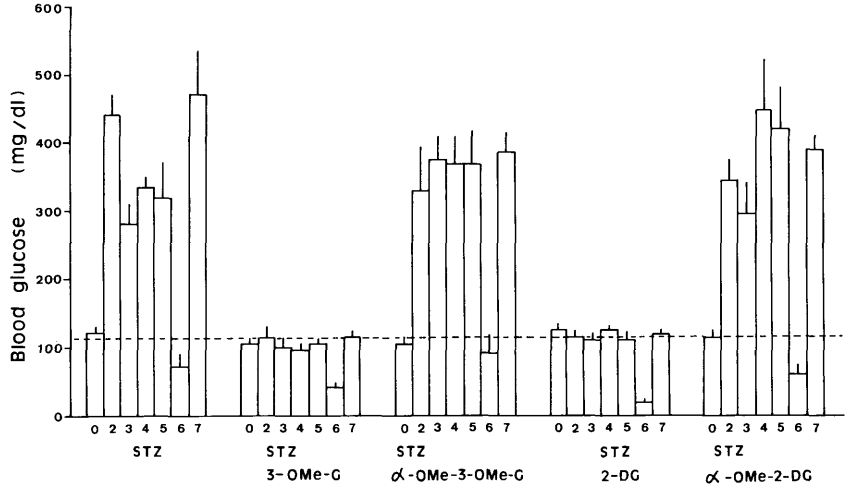



**FIGURE 3. Absence of protective effects of the α-methyl-glucosides of 3-OMe-glucose (α-OMe-3-OMe-G, 5 mmol/kg) and 2-deoxyglucose (α-OMe-2-DG, 10 mmol/kg) against STZ (60 mg/kg)-induced hyperglycemia. For comparison, the effects of 3-OMe-glucose (3-OMe-G, 6.9 mmol/kg) and 2-deoxyglucose (2-DG, 10 mmol/kg) are also shown. Numbers under columns indicate days after injection of the compounds. The glucose levels on day 0 are the initial levels. The levels on day 6 are those after starvation for 24 h. The broken line indicates the average normal blood glucose level. Six rats were used for each experiment.**

TABLE 1
Absence of diabetogenic activity of $C_1$-substituted STZ analogues

| STZ analogues | Dose (mg/kg) | Blood glucose (mg/dl) | N |
|---|---|---|---|
| STZ | 60 | 429 ± 49 | 6 |
| $C_1$-β-MNU-STZ | 120 | 120 ± 7 | 3 |
| | 180 | 109 ± 3 | 3 |
| β-OMe-STZ | 60 | 118 ± 12 | 5 |
| α-OMe-STZ | 120 | 117 ± 2 | 6 |
| | 150 | 105 ± 9 | 6 |
| STZ (60 mg/kg) plus α-OMe-STZ | 60 | 414 ± 58 | 6 |
| | 120 | 378 ± 109 | 6 |
| | 150 | 449 ± 80 | 6 |

MNU: Methylnitrosourea.
Blood glucose values are means ± SD on day 7 after injections.

was added dropwise and the whole mixture was stirred for 1 h at 5°C. The reaction mixture was then saturated with $(NH_4)_2SO_4$ and extracted twice with a mixture of ethylacetate and tetrahydrofuran (1:8). The organic layer was dried over $MgSO_4$ and concentrated. The residual oil was purified by column chromatography using silica gel (solvent: chloroform/ethylacetate/methanol, 6/2/1) to give 0.30 g of the α-anomer of the final compound (3) and 0.75 g of the corresponding mixtures.

## RESULTS

**Protective effects of glucose analogues against STZ.** As shown in Figure 1, 3-OMe-G injected i.v. simultaneously with STZ (60 mg/kg) caused dose-dependent protection of B-cells from the toxicity of STZ. Fifty percent protection was observed at about 0.7 mmol/kg 3-OMe-G, and with doses of >2.1 mmol/kg, protection was complete. 2-DG also had a protective effect, but less than that of 3-OMe-G, causing complete protection at 10 mmol/kg (data not shown). These results were consistent with those of Ganda et al.,[4] but the doses for 50% protection were lower in our experiments. This difference was probably due to the fact that we injected glucose analogues and STZ simultaneously. Furthermore, N-acetylglucosamine, which is similar in structure to STZ between the sugar moiety and the urea carbon, caused almost complete protection against STZ at 6.9 mmol/kg, although its pattern of protection was different from those of 3-OMe-G and 2-DG as shown in Figure 2. This compound has not previously been reported to inhibit STZ.

On the other hand, the α-methylglucosides of 3-OMe-G and 2-DG at doses of 5 and 10 mmol/kg, respectively, did not protect B-cells from STZ (Figure 3). The levels of blood glucose on day 6 are those after fasting for 24 h. In our separate experiments, it was shown that the levels of blood glucose of STZ-diabetic rats maximally increased within several hours after feeding and then sharply decreased, reaching normal or subnormal ranges after fasting for 24 h.

TABLE 2
Physical data of 3-OMe-streptozocin (α-anomer)

| | | | |
|---|---|---|---|
| Pale yellow crystals, m.p. 93° dec. | | | |
| $[\alpha]_D^{20}$ + 101.6° (c = 0.9, MeOH) | | | |
| IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3480, 3380, 3300, 1730, 1530, 1130, 1070 | | | |
| NMR $d_6$-DMSO | | | |
| 3.11 3H, s, $NCH_3$ | 3.44 3H, s, $OCH_3$ | | |
| 4.46 1H, t, J = 6 Hz, OH | | | |
| 5.08 1H, d-d, H-1 ◊ | 5.11 d, J = 3.5 Hz in $D_2O$ | | |
| 5.17 1H, d, J = 6 Hz, OH | 6.71 1H, d, J = 5 Hz, OH | | |
| 8.17 1H, d, J = 9 Hz, NH | | | |
| Analysis | C | H | N |
| Calculated | 37.50 | 6.25 | 14.58 |
| Found | 37.39 | 6.45 | 14.64 |

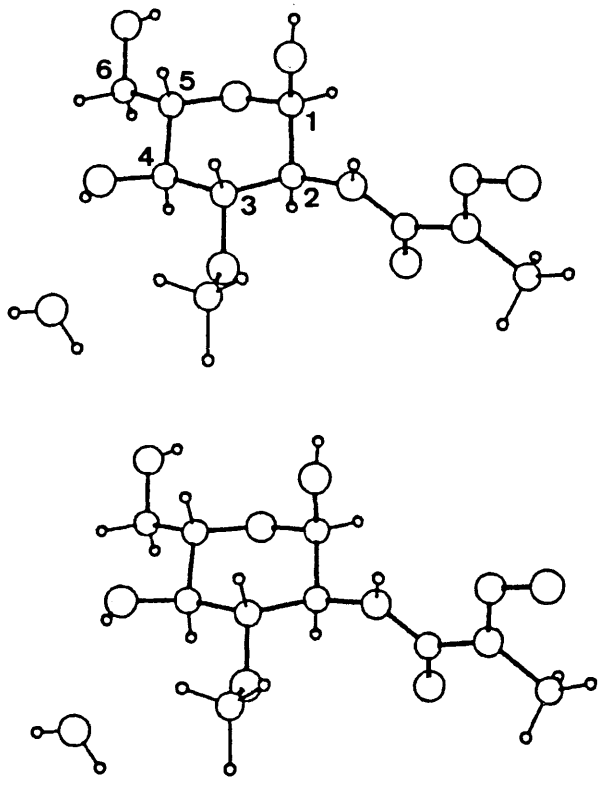



**FIGURE 4. Stereoview of the structure of 3-OMe-streptozocin (3-OMe-STZ). Crystal data: $C_9H_{17}N_3O_7 \cdot H_2O$, M = 297.27, trigonal, $P_{31}$ or $P_{32}$, a = 11.0963(5), c = 9.9300(9)Å, V = 1058.9(1)Å$^3$, Dc = 1.398 g/cm$^3$, Z = 3. Single crystals of 3-OMe-STZ were obtained from water by slow evaporation. The intensity data were collected using an automated four-circle diffractometer (Rigaku AFC-5). Of the total of 1206 independent reflections, 1109 had intensities above the 2.67σ (I) level and these were used for structure determination. The structure was solved by direct methods using MULTAN80 and refined by the block-diagonal, least-squares method with anisotropic temperature factors for nonhydrogen atoms and with isotropic factors for hydrogen atoms. The final value was 0.053.**

**Absence of diabetogenic effect of $C_1$-substituted STZ derivatives.** Results are summarized in Table 1. At doses of 60 mg/kg or more, $C_1$-β-methylnitrosourea-STZ and β-OMe-STZ were not diabetogenic. These results imply that $C_1$-β substitution sterically prevents the interaction or binding of the sugar moieties of these compounds with B-cell membranes.

α-OMe-STZ was also not diabetogenic even at higher doses. Injection of α-OMe-STZ (60, 120, and 150 mg/kg) with STZ had no appreciable influence on the effect of STZ. These results are consistent with the findings described above that α-OMe-3-OMe-G and α-OMe-2-DG had no protective effects. Therefore, there must be no steric hindrance around the oxygen atom of the glucopyranose ring for the binding of effective molecules to the hypothetical sugar recognition site of B-cell membranes.

**Properties of 3-OMe-STZ.** Assuming that 3-OMe-G binds competitively with STZ to the recognition site,[9] we synthe-

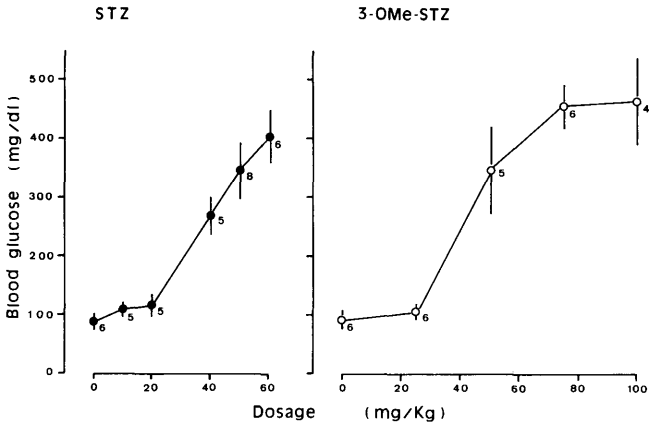



**FIGURE 5. Dose-response curve of a new compound, 3-OMe-streptozocin (3-OMe-STZ), compared with that of streptozocin (STZ). Both compounds were injected i.v. and blood glucose was determined 7 days later. Numbers by the symbols indicate the number of rats.**

sized 3-OMe-STZ expecting its positive diabetogenic effect. Physical data of the α-anomer of 3-OMe-STZ are summarized in Table 2. The chemical structure of this compound was determined from these data and also from x-ray crystallographic analysis (Figure 4). It was proven by thin-layer chromatography that the new compound was stable for at least 6 mo at −20°C.

**Diabetogenic effect of 3-OMe-STZ.** The degree of diabetes in rats afforded by 3-OMe-STZ was compared with that of STZ by employing various doses of these two compounds. As shown in Figure 5, the diabetogenic potency of 3-OMe-STZ was almost the same as that of STZ, as the mean blood glucose dose-response curves of these compounds were similar to each other.

## DISCUSSION

Under our experimental conditions, the dose of 3-OMe-G causing 50% protection against STZ (60 mg/kg) was about 0.7 mmol/kg. This dose is three times the diabetogenic dose of STZ on a molar basis. The protective effect can be explained by competition of 3-OMe-G with the sugar moiety of STZ for a recognition site on islet B-cells, judging from the following experimental results: (1) the protective effect of 3-OMe-G was dose dependent, (2) 2-DG and N-acetylglucosamine were also protective, (3) α-OMe-STZ was not diabetogenic, and (4) neither α-OMe-3-OMe-G nor α-OMe-2-DG had any protective effect. Furthermore, as known[4] and also confirmed in this work (data not shown), α- and β-methyl glucoside did not have protective effects. The β-anomer of STZ is reported to be less active than the α-anomer.[10] The $C_2$- and $C_4$-epimer types of STZ derivatives, whose sugar moieties are replaced by mannose (V) and galactose (VI), respectively, have been reported to be nondiabetogenic.[11] Therefore, the hypothetical recognition site seems to recognize the glucose structure specifically. In $C_3$-substituted compounds, such as 3-OMe-G, the original hydroxyl group at $C_3$ extends toward the outer-equatorial side of the glucopyranose ring remote from the ring-membered oxygen atom. Thus, the substitution of a small group, such as a methyl radical at $C_3$, is of less importance for the recognition site. This could be the reason that 3-OMe-G had a potent, positive, protective effect against STZ.

If there is a recognition site for the sugar moiety of STZ on B-cell membranes, we thought that 3-OMe-STZ should be diabetogenic, because the sugar portion of the compound, like 3-OMe-G, can compete with STZ for the hypothetical site. As expected, 3-OMe-STZ was actually diabetogenic and its potency was almost equivalent with that of STZ. The effectiveness of this new compound seems good evidence for the presence of a glucose recognition site on islet B-cell membranes. An interesting problem for future study is whether this site plays any role in the regulation of insulin secretion due to the blood glucose level.

## ACKNOWLEDGMENTS

The authors thank K. Aoe and K. Date of Tanabe Seiyaku Co. Ltd. for performing x-ray crystallographic analyses.

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