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NEW DEOXYNOJIRIMYCIN DERIVATIVES AS POTENT INHIBITORS OF INTESTINAL α-GLUCOHYDROLASES.

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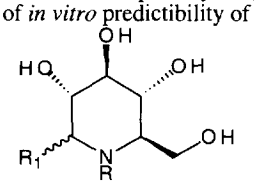
Abstract New N-alkyl, alkenyl and benzyl substituted DNJ derivatives incorporating a silicon atom in the substituent were synthesised. Kinetic parameters (K_i, $t_{1/2}$) for inhibition of rat intestinal α-glucohydrolases as well as human lysosomal α-glucosidases were measured. New DNJ derivatives are potent and selective inhibitors of intestinal α-glucohydrolases. © 1997, Elsevier Science Ltd. All rights reserved.

Inhibitors of intestinal α-glucohydrolases (sucrase, isomaltase, glucoamylase) constitute a new class of potentially beneficial oral drugs for the treatment of Diabetes Mellitus.
They act as potent antihyperglycemic agents by slowing down carbohydrate digestion to monosaccharides, thus delaying their absorption and significantly reducing the glycemic peak response to a meal [1,2].

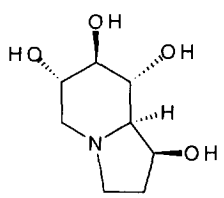
A large number of azasugars, generic name for molecules in which the oxygen of a natural sugar is substituted by a nitrogen, have been widely studied as glycosidase inhibitors.[2,3,4] Deoxynojirimycin (DNJ) **2** and a series of derivatives thereof have been reported to be particularly potent *slow binding* inhibitors of intestinal sucrase and isomaltase.[5,6,7] DNJ is the deoxy derivative of nojirimycin **1**, an aza analog of glucopyranose, which is a relatively poor inhibitor of intestinal α-glycosidases.[5]
Another natural compound castanospermine **3**, structure of which can be related to DNJ, was shown to be a potent *slow tight binding* inhibitor of rat small intestine sucrase and isomaltase by Danzin and collaborators in 1987 [8].These authors speculated that this quite unique property was due to the presence of a tertiary amine group. To eventually corroborate this hypothesis N-methyl DNJ **4** was assayed for *slow binding* inhibition of sucrase and isomaltase and, as expected, the k_{off} values, i.e the rate of dissociation of the inhibitor from the enzyme, for sucrase and isomaltase were found to be respectively 16 and 22 fold smaller than the corresponding k_{off} values of DNJ. [9]

$$E + I \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} EI \qquad k_{off} = t_{1/2} / 0.693$$

Moreover, studies of *in vivo* potency of glucosidase inhibitors suggest that k_{off} value may be the most important factor of *in vivo* predictibility of *in vivo* potency [2].



1 R=H,R_1=OH Nojirimycin
2 R,R_1=H Deoxynojirimycin (DNJ)
3 R=CH_3,R_1=H (N-methyl DNJ)



4 Castanospermine

For further corroboration of this hypothesis, new N-substituted DNJ derivatives were designed and their *in vitro* properties evaluated.
N-substituted DNJ bearing straight linear alkyl chain (C1 to C12) as well as alkyl chain bearing oxygen atom have been reported in literature[4,5,6], suggesting a lipophilic pocket in the enzyme active site.This prompted us to introduce a silicon atom in the substituting chain.[10a)]

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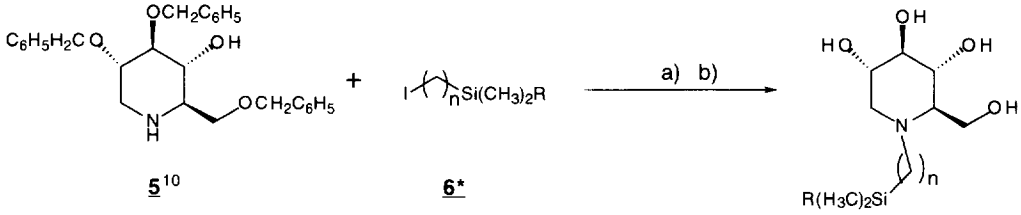
A first series of N-alkylsilyl DNJ was synthesised as shown in the following scheme: [10b]



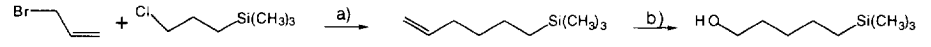
5[10] **6***

n=1: R=CH_3 **7**, R=C_3H_7 **8**, R=C_6H_5 **9**
n=3: R=CH_3 **10**, R=C_6H_5 **11**
n=4: R=CH_3 **12** R=C_6H_5 **13**
n=5: R=CH_3 **14**

Conditions: a) 2eq of NEt_3 or DIEA,2eq of **6**, DMF 80° overnight under argon atmosphere; b) CH_3OH/HCOOH 9/1-5%Pd/C under argon RT overnight; Yields range from 40 to 65% (not optimized) for 2 steps.

* If not commercially available the iodide is prepared either from the corresponding chloride (NaI excess, Acetone under reflux) or from the corresponding alcohol (a) MsCl NEt_3, CH_2Cl_2 b) MgI_2, Et_2O)

5-trimethylsilyl pentanol **15** was prepared in 2 steps from 3-chloropropyl trimethylsilane and allyl bromide.

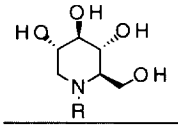


15

Conditions: a) Mg powder, THF reflux 14 h (50% yield) b)O_3 CH_2Cl_2-EtOH 1:1 - $NaBH_4$ in EtOH (60%yield)

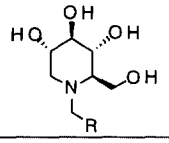
In vitro activities of compounds **7**-**14** on intestinal α-glucosidases were determined using purified preparation of sucrase-isomaltase complex and of glucoamylase from rat small intestine[8a, 11]. Kinetics parameters: K_i, the inhibition constant, and $t_{1/2}$, the time for half reactivation, were calculated as described by Danzin and collaborators[8].

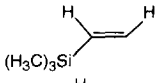
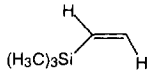
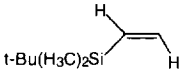
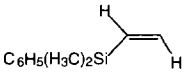
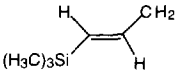
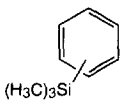
Table 1 *In vitro* potencies of N-Alkylsilyl DNJ derivatives



		SUCRASE		ISOMALTASE		GLUCOAMYLASE	
Substituent R	Cpd	Ki (μM)	$t_{1/2}$ (h)	Ki (μM)	$t_{1/2}$ (h)	Ki (μM)	$t_{1/2}$ (h)
- CH_2-$Si(CH_3)_3$	**7**	0.15	0.08	3.3	0.5	2.5	0.1
- CH_2-$Si(CH_3)_2C_3H_7$	**8**	0.11	0.13	7	0.25	0.9	0.1
- CH_2-$Si(CH_3)_2C_6H_5$	**9**	0.16	0.08	3.5	0.33	2.6	0.07
- $(CH_2)_3$-$Si(CH_3)_3$	**10**	0.008	4	0.12	4	0.05	1.4
- $(CH_2)_3$-$Si(CH_3)_2C_6H_5$	**11**	0.017	1.8	0.027	6	0.01	6.4
- $(CH_2)_4$-$Si(CH_3)_3$	**12**	0.016	0.5	0.04	4.8	0.12	0.2
- $(CH_2)_4$-$Si(CH_3)_2C_6H_5$	**13**	0.039	0.33	0.018	8	ND	ND
- $(CH_2)_5$-$Si(CH_3)_3$	**14**	0.033	0.3	0.016	12	0.07	0.4

Table 2 *In vitro* potencies of N-Alkenyltrialkylsilyl and N-benzyltrimethylsilyl DNJ derivatives



		SUCRASE		ISOMALTASE		GLUCOAMYLASE	
Substituent R	Cpd	K_i(μM)	$t_{1/2}$(h)	K_i(μM)	$t_{1/2}$(h)	K_i(μM)	$t_{1/2}$(h)
	**16**	0.35	0.24	0.64	0.9	ND	ND
	**17**	0.00015	180	0.1	8	0.0023	28
	**18**	0.01	13	0.39	3.5	0.087	2.0
	**19**	0.003	14	0.05	16	0.004	16
	**20**	0.22	0.1	ND	ND	ND	ND
	o **21**	8.5	0.12	4	0.11	10	0.18
	m **22**	0.055	3.5	11.5	0.13	1	0.5
	p **23**	0.064	3	0.83	2	0.028	5.5

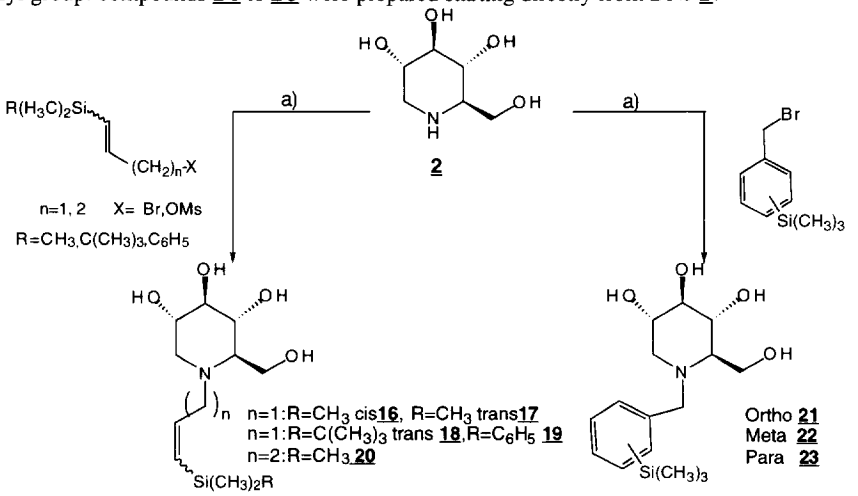
Data presented in table 2 clearly point out that not only the relative position but also the spatial orientation of the trialkyl silyl group is determinant for *in vitro* activity: the *trans* derivative **17** being the most potent *slow tight binding* inhibitor of intestinal sucrase ever reported whereas the *cis* analogue **16** is only a *slow binding* inhibitor with quite low potency.
Substitution of a methyl of the TMS in **17** by either a terbutyl (**18**) or a phenyl (**19**) group decreases somewhat potency as evidenced by $t_{1/2}$ values. As distinct from **17**, **20** its higher homologue is not a *tight binding* inhibitor of sucrase.

In the trimethylsilylbenzyl series the *ortho* derivative which can be related to the *cis* derivative **16** is indeed a poor inhibitor, the para derivative **23** being the best inhibitor in the series, but compared to **17** its potency is 10 and 100 times lower for sucrase and glucoamylase respectively.

Whereas our initial goal was the design of potent long-lasting or quasi-irreversible inhibitors of intestinal α-glucosidases, selectivity of the compounds toward the target enzymes was also an important feature; of particular concern was the inhibitory potential of those new DNJ derivatives toward liver lysosomal α-glucosidases.

All new DNJ derivatives are potent *tight binding* inhibitors of intestinal α-glucosidases with K_i values in the submicromolar to nanomolar range. Interestingly, inhibitory potency and $t_{1/2}$ values can be related to chain length and to the relative position of the silicon atom in the chain: 5 atom chain length and 3 carbon atom distance between the nitrogen of DNJ and silicon being the optimum (compare data obtained for cpd **8** and **10**).

Taking into account those first results, a second series of N-substituted DNJ derivatives was designed and evaluated *in vitro*, silicon atom being either included in an unsaturated carbon chain or part of a substituent of a benzyl group: compounds **16** to **23** were prepared starting directly from DNJ **2**.[12]

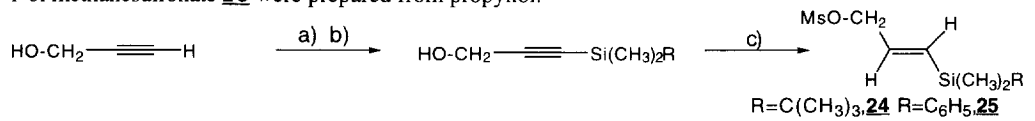


Conditions: a) 2eq of alkylating agent, 2eq of DIEA in DMF at 70 to 80°C for 12 to 20 h under argon atmosphere, yields range from 50 to 75% (not optimised).

Trimethylsilyl benzyl bromide derivatives were synthesised from the corresponding bromotoluene (BuLi, TMSCl -78°C in THF; NBS, CCl_4, 50% overall yield) [13].

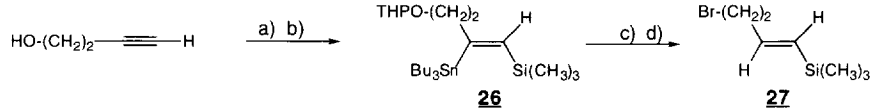
Pure (E)and (Z) 3-trimethylsilyl-2-propen-1-ol methanesulfonate were obtained as previously described from the corresponding pure isomeric alcohol[14].

(E)-3-(t-Butyldimethylsilyl)-2-propen-1-ol methanesulfonate **24** and (E)-3-(phenyldimethylsilyl)-2-propen-1-ol methanesulfonate **25** were prepared from propynol.



Conditions: a) 2eq BuLi, 2eq $ClSi(CH_3)_2R$, THF; b)Distannoxane cata. CH_3OH[15]; c) Red-Al Et_2O, MsCl-NEt_3 in CH_2Cl_2.

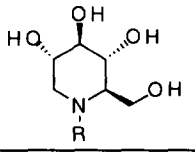
Pure (E)-4-trimethylsilyl-3-buten-1-bromide **27** was obtained in good yield from 3-butyn-1-ol as depicted below.

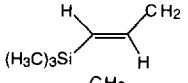
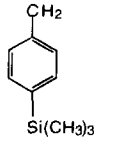


Conditions: a) DHP, PPTS, CH_2Cl_2; b) Bu_3Sn-$Si(CH_3)_3$, Tetrakis(triphenylphosphine)palladium in THF 70% yield (cpd **26** was previously reported in the litterature [16a]) c) CH_3Li in THF 90% yield [16b] d) $P(C_6H_5)_3$, CBr_4 in CH_2Cl_2 74% yield.

Inhibition of this enzyme could result upon long term treatment in a condition similar to the human glycogen storage disease known as Pompe disease.[17] Therefore we measured the *in vitro* inhibitory properties of our most potent intestinal disaccharidase inhibitors toward human lysosomal α-glucidases.[18]

Table 3 Inhibition of human lysosomal α-glucosidases by DNJ derivatives



		SUCRASE		LYSOSOMAL α-GLUCOSIDASE		SELECTIVITY RATIO
Substituent R	Cpd	Ki(μM)	$t_{1/2}$(h)	Ki(μM)	$t_{1/2}$(h)	$\frac{K_i \text{ Lys Glyco}}{K_i \text{ Sucrase}}$
H	**DNJ 2**	0.024	0.08	0.15	ND	6
$-CH_3$	**Me-DNJ 3**	0.02	1.7	0.42	3.5	21
$-(CH_2)_3-Si(CH_3)_3$	**9**	0.008	4	2.5	0.4	310
	**17**	0.00015	180	1	1.5	6700
	**23**	0.064	3	2.6	0.2	41

Results presented in table 3 show that the new potent N-substituted alkyl, alkenyl or benzyl trimethylsilyl DNJ derivatives are rather poor inhibitors of human lysosomal α-glucosidases

In conclusion, N-substituted DNJ derivatives bearing silicon atom in their side chain appear to be potent inhibitors of intestinal disaccharidases and the inhibition is of « *slow-tight-binding* » type, anticipating a long-lasting inhibition of the enzymes *in vivo*.
Moreover, compared to DNJ and Me-DNJ, the compounds reported here present a better selectivity profile.[8b]

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