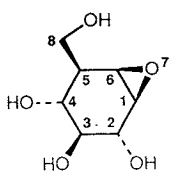
A FAMILY OF CYCLOPHELLITOL ANALOGS: SYNTHESIS AND EVALUATION

Sir:

Cyclophellitol (**1**) was isolated from culture filtrates of mushroom, *Phellinus* sp.[1], and found to be a highly specific and effective irreversible inactivator of β-glucosidases[2,3]. It is generally believed that the flattened half-chair conformation of the glycosyl intermediate is important for transition state binding by the enzyme[4,5]. The ground-state conformation of cyclophellitol (**1**) resembles the flattened half-chair conformation. Therefore, it is anticipated that the cyclophellitol analogs would have a variety of glycosidase-inhibitory activities. Recently, we have synthesized 1,6-*epi*-cyclophellitol (**2**)[6,7], the α-manno type analog **3**[8], and the aziridine analog **4**[8] (7-azabicyclo[4.1.0]heptane derivative), together with cyclophellitol (**1**) itself[7,9]. In a limited inhibitory activity study[6~9], it was shown that the glycoside-cleaving enzymes recognized the configurations of these compounds. It is noteworthy that the aziridine analog **4** showed a high inhibitory activity against almond β-glucosidase of IC_{50} 0.22 μg/ml[8]. To better understand the structure-inhibition relationship, we synthesized another aziridine analog **5**, the thiirane analogs **6** and **7**, the *N*-alkyl aziridine analogs **8**~**10**, and the *N*-acyl aziridine analog **11**. A preliminary glucosidase inhibitory activity study was also performed.

The synthesis of **5** began with natural cyclophellitol (**1**) according to the same procedures used for the synthesis of **4**[8]. Cyclophellitol (**1**) was benzylated with BnBr and NaH in DMF at 25°C for 0.5 hour to give the tetra-*O*-benzyl derivative **12** in 90% yield. Treatment of **12** with NaN_3 in DMF (110°C, 12 hours) afforded **13** and **14** in 27% and 41% yield, respectively: **13**: $[\alpha]_D^{25}$ +15° (*c* 0.34, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 1.90 (1H, m, 5-H†), 4.02 (1H, s, OH); *Anal* Calcd for $C_{35}H_{37}N_3O_5$: C 72.52, H 6.43, N 7.25. Found: C 72.92, H 6.97, N 6.86. **14**: $[\alpha]_D^{25}$ −2.4° (*c* 0.76, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 1.48 (1H, dddd, $J_{4,5}=J_{5,6}=10.8$ Hz, $J_{5,8}=J_{5,8'}=2.0$ Hz, 5-H†), 2.51 (1H, d, $J=2.0$ Hz, OH), 3.49 (1H, ddd, $J_{1,2}=J_{1,6}=9.8$ Hz, 1-H), 3.70 (1H, dd, 6-H); *Anal* Calcd for $C_{35}H_{37}N_3O_5$: C 72.52, H 6.43, N 7.25. Found: C 72.59, H 6.31, N 7.03. The ^1H NMR spectrum of **14** clearly indicated ($J_{1,2}=J_{1,6}=9.8$ Hz, $J_{5,6}=10.8$ Hz) that the C-1 hydroxyl group and the C-6 azide group of **14** are oriented equatorially. Since the one-step procedure (Ph_3P, toluene, 110°C, 0.5 hour)[8] to obtain the aziridine **15** from a mixture of **13** and **14** did not succeed, a three-step procedure was necessary for this transformation: i) MsCl, pyridine, 25°C, 12 hours, ii) Ph_3P, THF, 25°C, 0.5 hour, then H_2O, 25°C, 12 hours, iii) NaOMe, MeOH, 25°C, 1.5 hours, 40% overall yield. Finally, de-*O*-benzylation of **15** (Li, liq NH_3, ether, −78°C, 1 hour) afforded the aziridine analog **5** in 60% yield: $[\alpha]_D^{25}$ +28° (*c* 0.12, H_2O); ^1H NMR (270 MHz, D_2O, DOH=4.80) δ 1.94 (1H, m, 5-H), 2.41 (1H,



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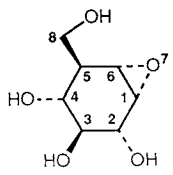

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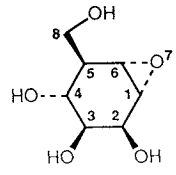

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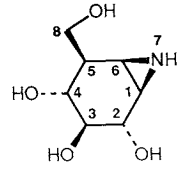

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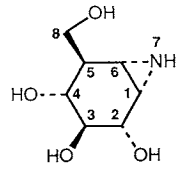

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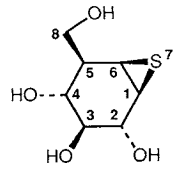

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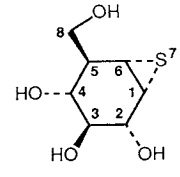

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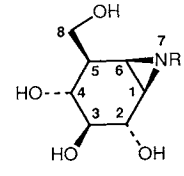
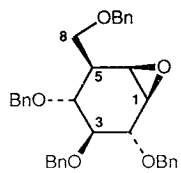


8 R=Me
9 R=Et
10 R=*n*-Bu
11 R=butyryl

† The carbon-numbering protocol of **13** and **14** anticipates conveniently the construction of the aziridine analog **5**.



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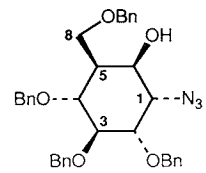

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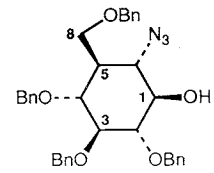

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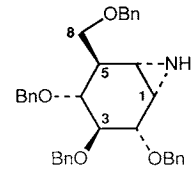

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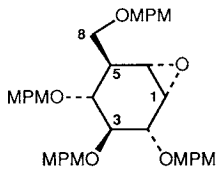

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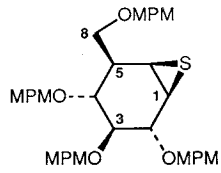

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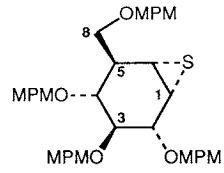

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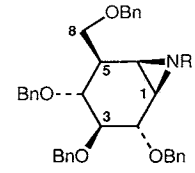

19 R=H
20 R=Me
21 R=Et
22 R=*n*-Bu

d, $J_{5,6}=0$ Hz, $J_{1,6}=8.2$ Hz, 6-H), 2.66 (1H, dd, $J_{1,2}=3.8$ Hz, 1-H), 3.06~3.19 (2H, m, 3- and 4-H), 3.73 (1H, dd, $J_{gem}=11.0$ Hz, $J_{5,8}=6.8$ Hz, 8-H), 3.83 (1H, dd, $J_{2,3}=8.6$ Hz, 2-H), 3.92 (1H, dd, $J_{5,8'}=4.0$ Hz, 8'-H).

The thiirane analogs **6** and **7** were prepared as follows. 1,6-*epi*-Cyclophellitol (**2**) was protected as its tetra-*O*-methoxybenzyl ether **16** in 60% yield by treatment with 4-methoxybenzyl (MPM) chloride and NaH in DMF at 25°C for 20 hours. Thiirane formation[10] was realized by treatment of **16** with $Ph_3P=S$ and trifluoroacetic acid in benzene at 60°C for 48 hours to give **17** in 52% yield: $[\alpha]_D^{25}$ +73° (*c* 0.18, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 3.17 (1H, d, $J_{1,6}=6.2$ Hz, $J_{1,2}=0$ Hz, 1-H), 3.57 (1H, dd, $J_{5,6}=4.0$ Hz, 6-H); *Anal* Calcd for $C_{39}H_{44}O_8S$: C 69.62, H 6.59. Found: C 69.55, H 6.45. It was assumed by the proposed reaction mechanism[10] that the C1- and the C6-configurations were inverted under these conditions. Finally, de-*O*-methoxybenzylation of **17** (DDQ, CH_2Cl_2-MeOH-H_2O, 25°C, 12 hours)[11] afforded the thiirane analog **6** in 65% yield: $[\alpha]_D^{25}$ +102° (*c* 0.22, MeOH); ^1H NMR (270 MHz, CD_3OD) δ 2.31 (1H, m, 5-H), 3.09 (1H, d, $J_{1,6}=6.6$ Hz, $J_{1,2}=0$ Hz, 1-H), 3.15~3.25 (2H, m, 3- and 4-H), 3.52 (1H, dd, $J_{5,6}=4.0$ Hz, 6-H), 3.56 (1H, dd, $J_{gem}=10.4$ Hz, $J_{5,8}=9.0$ Hz, 8-H), 3.99 (1H, d, $J_{2,3}=7.6$ Hz, 2-H), 4.11 (1H, d, $J_{5,8'}=4.0$ Hz, 8'-H). In an analogous fashion, cyclophellitol (**1**) was transformed to **7** via **18** in 20% overall yield: **18**: ^1H NMR (270 MHz, $CDCl_3$) δ 3.08 (1H, dd, $J_{1,6}=6.8$ Hz, $J_{5,6}=2.0$ Hz, 6-H), 3.33 (1H, dd, $J_{1,2}=3.8$ Hz, 1-H), 3.77, 3.78, 3.80, 3.82 (each 3H, each s, 4 × OMe). **7**: $[\alpha]_D^{25}$ +110° (*c* 0.16, MeOH); ^1H NMR (270 MHz, CD_3OD) δ 2.28 (1H, m, 5-H), 3.16 (1H, dd, $J_{1,6}=6.0$ Hz, $J_{5,6}=0$ Hz, 6-H), 3.23 (1H, t, $J_{3,4}=J_{4,5}=10.0$ Hz, 4-H), 3.42 (1H, dd, $J_{1,2}=4.0$ Hz, 1-H), 3.49 (1H, dd, $J_{2,3}=8.4$ Hz, 3-H), 3.69 (1H, dd, $J_{gem}=10.8$ Hz, $J_{5,8}=6.6$ Hz, 8-H), 3.89 (1H, dd, $J_{5,8'}=4.0$ Hz, 8'-H), 4.02 (1H, dd, 2-H).

The *N*-alkyl aziridine analogs **8**~**10** were prepared from the tetra-*O*-benzyl aziridine derivative **19**[8]. **19** was subjected to the *N*-alkylation conditions (Me_2SO_4/K_2CO_3/DMF, 25°C, 15 hours, Et_2SO_4/K_2CO_3/DMF/50°C/48 hours, and *n*-BuI/K_2CO_3/DMF/50°C/20 hours, respectively) to afford **20**, **21** and **22** in 60%, 35% and 50% yield, respectively. De-*O*-benzylation (Li, liq NH_3, ether, −70°C, 1 hour) of the above compounds provided **8**, **9** and **10** in 96%, 75% and 70% yield, respectively: **8**: $[\alpha]_D^{25}$ +128° (*c* 0.12, MeOH); ^1H NMR (270 MHz, CD_3OD) δ 1.62 (1H, d, $J_{1,6}=6.4$ Hz, $J_{1,2}=0$ Hz, 1-H), 1.89 (1H, m, 5-H), 1.99 (1H, dd, $J_{5,6}=3.2$ Hz, 6-H), 2.33 (3H, s, NMe), 2.97 (1H, dd, $J_{3,4}=J_{4,5}=10.0$ Hz, 4-H), 3.08 (1H, dd, $J_{2,3}=8.0$ Hz, 3-H), 3.57 (1H, d, 2-H), 3.62 (1H, dd, $J_{gem}=J_{5,8}=10.6$ Hz, 8-H), 3.98 (1H, dd, $J_{5,8'}=5.0$ Hz, 8'-H). **9**: $[\alpha]_D^{25}$ +77° (*c* 0.12, MeOH); ^1H NMR (270 MHz, CD_3OD) δ 1.15 (3H, t, $J=6.8$ Hz, Me), 1.67 (1H, d, $J_{1,6}=6.4$ Hz, $J_{1,2}=0$ Hz, 1-H), 1.88 (1H, m, 5-H), 2.00 (1H, dd, $J_{5,6}=3.2$ Hz, 6-H), 2.11, 2.48 (each 1H, each m, NCH_2), 3.00 (1H, dd, $J_{3,4}=J_{4,5}=10.0$ Hz, 4-H), 3.10 (1H, dd, $J_{2,3}=8.0$ Hz, 3-H), 3.57 (1H, d,

Table 1. Glucosidase inhibitory activities of **5**~**11**. [IC_{50} (μg/ml) (I % at 100 μg/ml in parentheses)].

Enzyme tested	**5**	**6**	**7**	**8**	**9**	**10**	**11**
β-Glucosidase (almond)	32 (84)	(2)[a]	(8)	24 (82)	(18)	1.3 (98)	0.3 (95)[b]
α-Glucosidase (baker yeast)	(12)	(44)	(55)	(46)	(38)	(20)	(66)

[a] 30 μg/ml.
[b] 10 μg/ml.

2-H), 3.61 (1H, dd, $J_{gem}=J_{5,8}=10.0$ Hz, 8-H), 3.99 (1H, dd, $J_{5,8'}=4.0$ Hz, 8'-H). **10**: $[\alpha]_D^{25}$ +65° (*c* 0.33, MeOH); ^{1}H NMR (270 MHz, CD_3OD) δ 0.94 (3H, t, $J=7.0$ Hz, Me), 1.36, 1.56 (each 2H, each m, CH_2CH_2), 1.64 (1H, d, $J_{1,6}=6.4$ Hz, $J_{1,2}=0$ Hz, 1-H), 1.88 (1H, m, 5-H), 1.98 (1H, dd, $J_{5,6}=3.2$ Hz, 6-H), 2.16, 2.34 (each 1H, each m, NCH_2), 3.02 (1H, dd, $J_{3,4}=J_{4,5}=10.0$ Hz, 4-H), 3.10 (1H, dd, $J_{2,3}=8.0$ Hz, 3-H), 3.58 (1H, d, 2-H), 3.62 (1H, dd, $J_{gem}=J_{5,8}=10.0$ Hz, 8-H), 3.99 (1H, dd, $J_{5,8'}=4.6$ Hz, 8'-H). The *N*-butyryl analog **11** was directly prepared from **4** by treatment with butyryl chloride and triethylamine in MeOH at 25°C for 15 minutes in 65% yield: $[\alpha]_D^{25}$ +56° (*c* 0.26, MeOH); ^{1}H NMR (270 MHz, D_2O, DOH=4.80) δ 0.91 (3H, t, $J=7.0$ Hz, Me), 1.62 (2H, sextet, $J=7.0$ Hz, CH_2), 2.08 (1H, m, 5-H), 2.50 (2H, t, $J=7.0$ Hz, $COCH_2$), 2.86 (1H, d, $J_{1,6}=6.4$ Hz, $J_{1,2}=0$ Hz, 1-H), 3.17 (1H, dd, $J_{5,6}=2.4$ Hz, 6-H), 3.21 (1H, dd, $J_{3,4}=J_{4,5}=10.4$ Hz, 4-H), 3.34 (1H, dd, $J_{2,3}=8.4$ Hz, 3-H), 3.76 (1H, dd, $J_{gem}=11.0$ Hz, $J_{5,8}=8.4$ Hz, 8-H), 3.81 (1H, d, 2-H), 4.04 (1H, dd, $J_{5,8'}=4.4$ Hz, 8'-H).

The glycosidase inhibitory activities of **5**~**11** were generally assayed according to the method reported by SAUL *et al.*[12] and are shown in Table 1. The previous evaluation[6~9] of **1**, **2**, **3** and **4** revealed that the glycoside-cleaving enzymes recognized the configurations of these compounds including the epoxidic and the aziridinic configurations. On the contrary, the new aziridine analog **5** showed inhibitory activity only against almond β-glucosidase with an IC_{50} of 32 μg/ml (indeed, **5** was a weak inhibitor of baker yeast α-glucosidase, *Escherichia coli* β-galactosidase, and jack bean α-mannosidase, data not shown). These findings reflect that the inhibition mechanisms of the epoxide and the aziridine analogs are different. Neither the thiirane analog **6** nor **7** showed significant activities. Various *N*-alkyl derivatives of 1-deoxynojirimycin were shown to have different inhibition properties, especially anti-HIV activity[13]. Among **8**, **9** and **10**, the *N*-butyl aziridine analog **10** is a better almond β-glucosidase inhibitor (IC_{50} 1.3 μg/ml) than the *N*-methyl and *N*-ethyl derivatives. Furthermore, the *N*-butyryl analog **11** showed inhibitory activity against almond β-glucosidase of IC_{50} 0.3 μg/ml. These results suggest that the *N*-substituent may play a key role for inhibition. Further study along this line is now in progress.

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