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Stereospecific synthesis of highly substituted novel carbasugar as carbonic anhydrase inhibitors: decahydronaphthalene-1,2,3,4,5,6,7-heptol

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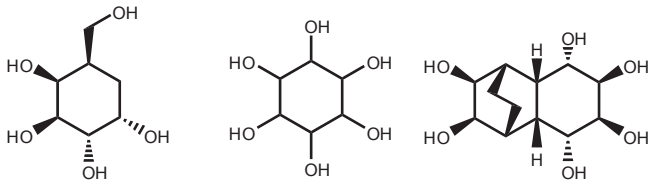
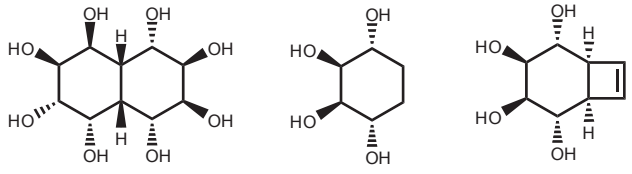
ABSTRACT

Decahydronaphthalene-1,2,3,4,5,6,7-heptol, a new polycyclitol, was synthesized starting from *p*-benzoquinone. An *endo* selective Diels–Alder cycloaddition between *p*-benzoquinone and 1-acetoxybutadiene followed by stereoselective reduction with $NaBH_4/CeCl_3 \cdot 7H_2O$ led to the formation of an allylic *cis*-diol. The formed diol was converted into its acetate with Ac_2O/pyridine, in a transformation that required inert atmosphere conditions to suppress a competing aromatization. Controlled oxidation by OsO_4 of two olefinic bonds followed by acetylation yielded the heptaacetate whose structure was established unequivocally via application of X-ray crystallographic methods. Removal of the acetate groups by NH_3 provided the target heptol. In addition, the carbonic anhydrase inhibitory potency of the title compound was investigated and it was shown to be a potent inhibitor compared to the standard CA inhibitors.

1. Introduction

Carbasugars **1**[1] are mimics of monosaccharides in biological systems in which the ring oxygen is replaced with a methylene group.[2] They have attracted a great deal of interest among organic and medicinal chemists due to their glycosidase inhibitory activity.[3] Glycosidase enzymes are involved in numerous biological processes and their inhibition has enormous potential for the treatment of many diseases. Furthermore, in connection with the carbasugars, the polyhydroxylated carbocyclics (particularly, bicyclic and tricyclic derivatives) have gained importance. Many analogues and structural variants of **1** and inositol **2** have been synthesized[4i,j] and the field remains extremely active.[4] Their biological activities, particularly glycosidase inhibition, have played a fundamental role in the development of new drugs.[5] Thus, the synthesis of polycyclitols **3** and **4** as new structural variants embodying the characteristic features present in **1** and **2** has recently been achieved.[6] In 1994 Billington and co-workers synthesized a series of tricyclic polyhydroxylated compounds, such as **3**[7] and reported that the biological activities of several of these tricyclic derivatives (stimulation of insulin secretion) were similar to conduritol A and E. In 2000 Mehta[8] and Ramesh reported the synthesis of fully hydroxylated bicyclic derivatives with decalin skeleton **4**, which may be regarded as a hybrid of two carbasugars. Consequently, the synthesis of carbasugars and their derivatives in a stereoselective manner have attracted much interest among chemists and biochemists owing to their ability to serve as potential glycosidase inhibitors.





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On the other hand, dihydroconduritol-A **5** was found and isolated by Zeying and Mingzhe from the plant *Toxocarpus* (from the Asclepiadaceae family), which grows in China and some areas of India.[9] They have named the naturally occurring compound as toxocarol and reported that this plant is being used in the treatment of fractures, contusions, ulcers, and cancers of the cervix, uterus, and lung.[9] Cyclitol preparation is challenging due to the dense stereochemistry of the hydroxylated carbon centers. Furthermore, analogues of these compounds are even more difficult synthetic targets due to additional hydroxyl groups. Recently, we have reported the stereospecific synthesis of some polyhydroxylated compounds.[10] In our previous studies, we had successfully used cyclooctatetraene for the stereospecific synthesis of bis-homoconduritol-A **6**[10] having a structure analogous with **5** as well as bis-homoconduritol-D and -F.

Carbonic anhydrase inhibitors (CAIs) are a class of pharmaceuticals used as anti-glaucoma agents, diuretics, antiepileptics, and in the management of mountain sickness, gastric, and duodenal ulcers, neurological disorders or osteoporosis. The carbonic anhydrases (CAs, EC. 4.2.1.1) represent a class of ubiquitous zinc-containing enzymes widespread in all living organisms, which classically participate in the maintenance of pH homeostasis in mammalians, catalyzing the reversible hydration of CO_2 in a two-step reaction to yield HCO_3^- and H^+.[11] Due to the important roles of CAs in higher vertebrates, compounds possessing CA inhibitory properties, mainly aromatic/heterocyclic sulfonamides (such as acetazolamide and zonisamide), and sulfamides incorporating sugar moieties have been used as drugs in the therapy of different pathologies such as bacterial infections, glaucoma, various neurological/neuromuscular disorders, epilepsy, acid–base disequilibria, or as diuretics.[11–13]

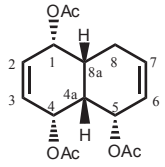
Herein, we report the stereoselective synthesis of a bicyclitol based on the decalin system, which can be regarded as an annulated dihydroconduritol-A or carbasugar represented by **4** as potential glycomimetics and also CA inhibitory potency of the molecules.

2. Results and discussion

The synthetic route that was followed for allylic *cis*-diol **10** started with an *endo* selective Diels–Alder cycloaddition between benzoquinone **7** and 1-acetoxybutadiene **8** and produced smoothly cycloadduct **9**[14] as the sole product in 80% yield. The allylic *cis*-diol **10** is a key intermediate for our synthesis and allows the stereoselective formation of a carbasugar product. The required allylic *cis*-diol **10** as the sole product was obtained by stereoselective reduction of the carbonyl groups with the $NaBH_4/CeCl_3\cdot 7H_2O$ system.[15] In our previous studies,[14a] the stereochemical course of the reduction was determined as *syn* according to the acetate group and it was later proved through X-ray analysis of the pentaacetate **14**. Without any purification, the crude diol was treated with CH_3COCl in methylene chloride to yield the corresponding triacetate **11**. Contrary to expectations, acetylation of **10** resulted in the formation of 1,4-diacetoxynaphthalene **12**[16] having an aromatic structure as well as the targeted triacetate **11** (Scheme 1).

For overcoming this potential challenge, we aimed to use the alternative acetylation methods. Thus, we performed a detailed study of the acetylation to find the optimal conditions. Acetylation of **10**, carried out with CH_3COCl in methylene chloride at room temperature (method A),[17] proceeded to give a mixture of compounds **11** and **12**. The mixture was separated by chromatography to yield **11** and **12** in 38:49% yield (1.72 g), respectively. As the second acetylation method, we used the acetic anhydride/pyridine system at room temperature for 15 h (method B)[10,14a,18] and the same mixture of **11**/**12** in a ratio of 42:46% yield (1.98 g) was obtained, respectively. However, treatment with acetic anhydride/CH_3COONa system at 90 °C for 12 h (method C)[19] did not give the expected result. Ratio of the purified products is 39:39% yield (1.82 g). Consequently, all attempts to obtain as the sole product compound **11** was not successful. Fortunately, when method B was used under an inert atmosphere (N_2), compound **11** was successively obtained as the sole product in 89% yield (Scheme 1). In the light of all these findings, one can conclude that the aromatization to compound **12** occurs readily during acetylation of allylic *cis*-diol **10** as an undesired product along with allylic *cis*-diacetate. Thus, the aromatized product **12** proceeded smoothly by loss of the acetoxy substituent (−OAc) at C(5) position through elimination.[20] In order to confirm this unexpected result, the acetylation of allylic *cis*-diol **10** was repeated, and consecutive yield of triacetate **11** was found as 85–89%.

The structures of **11** and **12** were assigned on the basis of ^1H and ^{13}C NMR spectra. Compound **11** showed the presence of three −OAc whereas **12** displayed a signal belonging to six aromatic protons and two OAc. Furthermore, ^{13}C NMR spectrum of **12** consisted of seven carbon resonances owing to mirror symmetry in the molecule. These results also clearly showed that compound **12** has an aromatic structure. In ^{13}C NMR of compound **11**, the carboxyl and olefinic carbons were observed at 171.0, 170.4, 170.2 ppm (C=O) and 129.8, 129.7, 126.3, 125.9 ppm (CH=CH), respectively.



11

In the ^1H NMR spectrum of **11**, irradiation of H-7 at δ 5.79 caused H-6 at δ 5.47 to turn from a doublet to a broad singlet. Irradiation of one of the geminal protons H-8 at δ 1.87, H-7 and H-6 protons showed a clear AB system, which H-7 at δ 5.79 turned from a multiplet to a doublet and H-6 at δ 5.47 also turned from a broad doublet to a doublet, respectively. These results clearly indicate that H-7 with H-8 protons and H-6 with H-7 are the neighboring protons with each other. Irradiation of H-4a at δ 2.58 caused the signal of H-5 at δ 5.40 to turn from a multiplet to a broad singlet. These results clearly indicate that H-5 with H-4a are the neighboring protons with each other.

Two double bonds in compound **11** should in the next step be *syn*-dihydroxylated for the synthesis of heptaacetate **16**. We first aimed at selective oxidation of only one of the two double bonds in **11** in a stereoselective manner. Thus, *cis*-dihydroxylation of compound **11** with catalytic OsO_4 in the presence of *N*-methylmorpholine *N*-oxide (NMO) as cooxidant in a mixture of H_2O/$(CH_3)_2CO$ (1:5) at room temperature gave the corresponding diol **13**, subsequent acetylation with Ac_2O and pyridine yielded the pentaacetate **14** as a single isomer in 85% yield (Scheme 2).

Careful examination of the reaction mixture did not reveal the formation of any other isomer and/or any aromatized product. As regards the observed stereoselectivity, we assumed that the cis-hydroxylation occurred from the *anti* position of the acetate due to the lower hindrance. The NMR spectroscopic studies did not allow the assignment of the exact configuration of the hydroxyl groups or acetate groups. X-ray analysis of pentaacetate **14** (Fig. 1) confirmed the structural assignment, and in particular the configurations of the reduction product **10** and cis-hydroxylation product **13**.

After we obtained the pentaacetate **14** successfully, we tried to synthesize heptol **17** using the same procedure as described above. cis-Dihydroxylation of compound **14** with catalytic OsO_4 and NMO at room temperature furnished a single stereoisomeric diol due to much greater steric accessibility of the *anti*-face of the double bond according to acetate groups. To avoid protecting-group migration, the product was converted into the heptaacetate **16** in 89% yield

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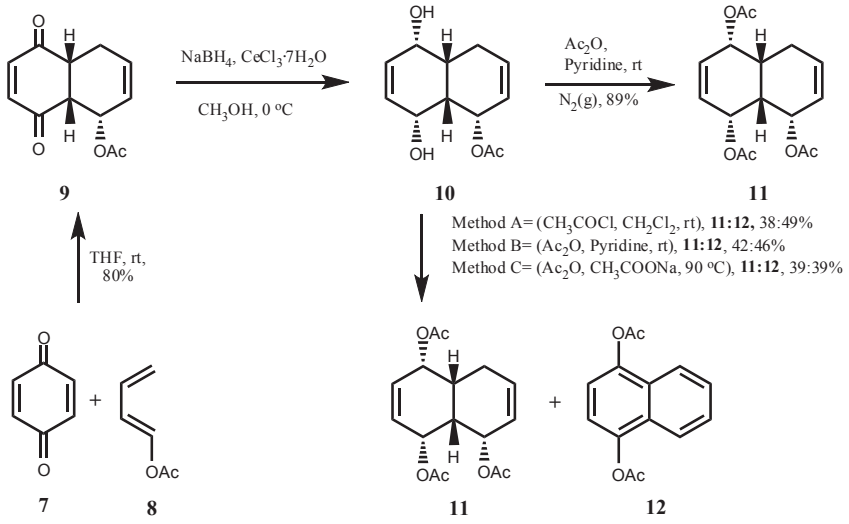
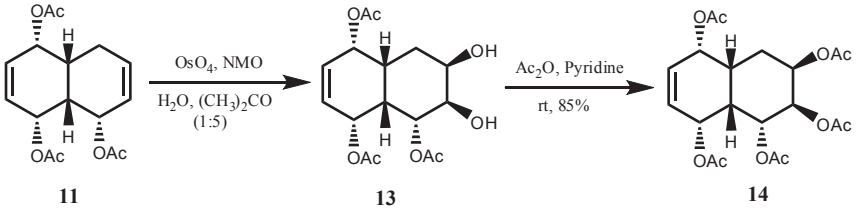


Scheme 1. Acetylation of *cis*-diol acetate **10**.



Scheme 2. Synthesis of pentaacetate **14**.

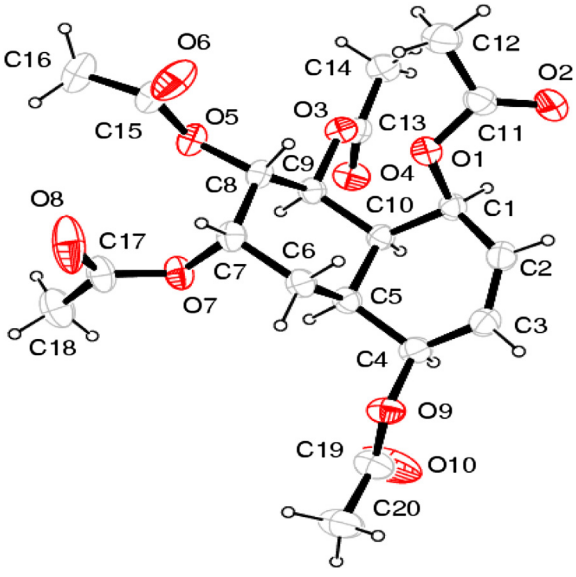
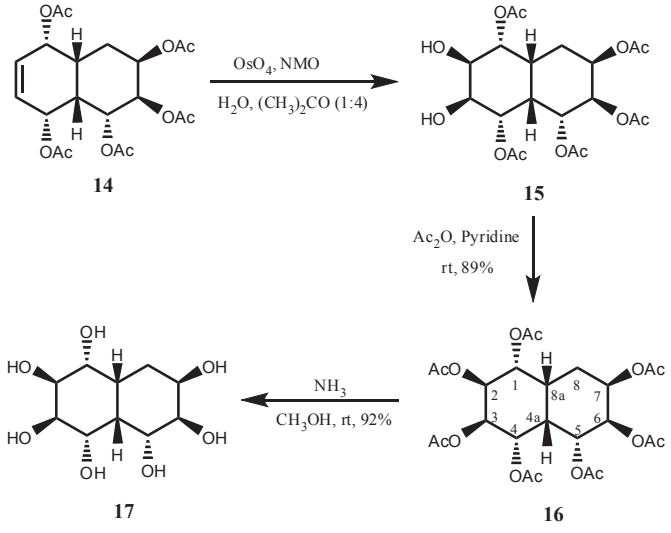


Fig. 1. ORTEP drawing of pentaacetate **14** with the atom-numbering scheme. Displacement ellipsoids are drawn at the 40% probability level.



Scheme 3. Synthesis of heptol **17**.

directly (Scheme 3). All analytical methods showed the formation of a single isomer. The NMR spectroscopic studies could not detect the exact configurations of the hydroxyl groups, but X-ray analysis of the heptaacetate **16** (Fig. 2) revealed the structural assignment, configurations of the heptaacetate **16** along with *cis*-hydroxylation product **15**, in particular.

The final step of our synthesis required removal of the acetate groups and this was accomplished with ammonia in methanol at room temperature. After recrystallization, the heptol **17** with dihydroconduritol-A configuration was obtained as a single isomer in 92% yield (Scheme 3). The structure of **17** was assigned on the basis of ^{1}H and ^{13}C NMR spectra. In particular, ^{13}C NMR spectrum of **17** consisted of 10 carbon resonances due to the asymmetry in the molecule and it was consistent with the proposed structure.

Inhibitory effects of compounds **17**–**19** including hydroxy functional group and AZA on enzyme activities were tested under in vitro conditions; K_i values were calculated from Lineweaver–Burk graphs and are given in Table 1.[21]

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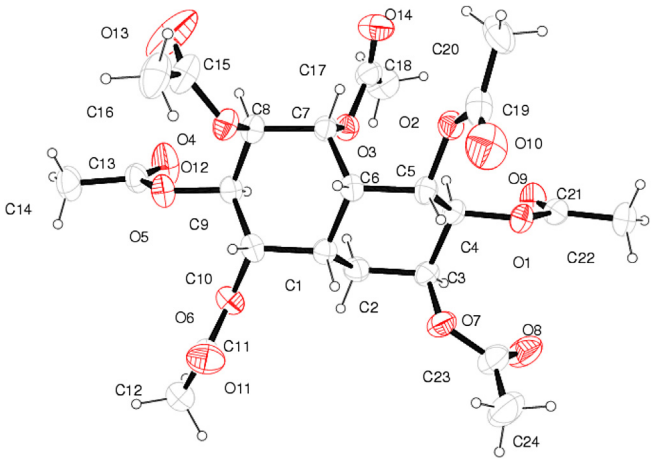
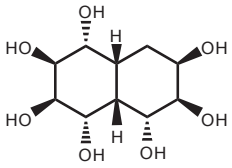
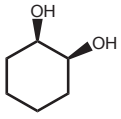
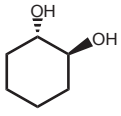
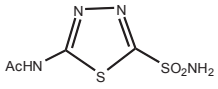


Fig. 2. ORTEP drawing of heptaacetate **16** with the atom-numbering scheme. Displacement ellipsoids are drawn at the 40% probability level.

Table 1
hCA I and hCA II inhibition data with compounds **17**–**19** and AZA

Compound	K_i (μM)	
	hCA I	hCA II
 **17**	17.4	1.13
 **18**	21.72[a]	1.84[a]
 **19**	134.6[a]	4.72[a]
 **AZA**	36.2[b]	0.37[b]

Mean from at least three determinations.
Errors in the range of 1–3% of the reported value (data not shown).
[a] From Ref. 22.
[b] From Ref. 23.

We report here the first study on the inhibitory effects of heptol **17** on the esterase activity of hCA I and II. The sulfonamide specific carbonic anhydrase inhibitor acetazolamide **AZA**, *cis*/*trans* diols **18** and **19** have been used for comparison. It was determined that heptol **17** had inhibitory effect on hCA I and II isozymes due to the presence of hydroxy functional groups. The inhibitory effect of heptol **17** (carbasugar) on these isozymes was stronger than *cis*/*trans* diols **18** and **19**.[22] When compared with AZA[23] (mainly used as an anti-glaucoma agent) inhibitory effect of heptol **17** on hCA I was stronger, but weaker than hCA II. Thus in contrast to *cis*/*trans* diols **18** and **19**, heptol **17** (as a carbasugar derivative) acts as much more efficient inhibitors against both isozymes.

3. Conclusion

In conclusion, we have achieved the synthesis of novel carbasugar starting from commercially available *p*-benzoquinone. An *endo* selective Diels–Alder cycloaddition between *p*-benzoquinone and 1-acetoxybutadiene followed by stereoselective reduction with $NaBH_4/CeCl_3\cdot 7H_2O$ led to the formation of an allylic *cis*-diol. The diol functionalities were acetylated by Ac_2O/pyridine system under nitrogen gas. Oxidation of two double bonds of triacetate with OsO_4 followed by acetylation produced compound heptaacetate whose exact configurations were determined by X-ray diffraction analysis. Controlled removal of the acetate groups by NH_3 furnished the desired new carbasugar. Compound **17** represents the first member of a new class of structures comprising seven hydroxyl groups on a *cis*-decalin scaffold. We envisage that this type of compound may have important biological activities and could be subject to additional pharmacological research.

4. Experimental section

4.1. General

Melting points were determined on a Buchi 539 capillary melting apparatus and are uncorrected. IR was obtained from KBr or film on a Mattson 1000 FT-IR spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded on 400 (100) MHz Varian spectrometer and are reported in δ units with $SiMe_4$ as internal standard. TLC was performed on E. Merck Silica Gel 60 F_{254} plate (0.2 mm). All column chromatography was performed on silica gel (60 mesh, Merck). EIMS spectra were recorded on a Thermo-Fingnigan and Perkin–Elmer Clarus 500 GS/MS analyzer. Elemental analyses were carried out on a Carlo Erba 1108 model CHNS-O analyzer.

4.2. 1-Acetoxy-5,8-dioxo-1,4,4a,5,8,8a-hexahydronaphthalene (9)

It was prepared according to the procedure described in the literature.[14]

4.3. (±)-(1*S**,4*R**,4a*R**,5*S**,8a*R**)-1,4,4a,5,8,8a-Hexahydronaphthalene-1,4,5-triyl triacetate (11) and 1,4-diacetoxynaphthalene (12)

4.3.1. Method A. The reduction of monoacetoxydiketone **9** (1.60 g, 7.27 mmol) was carried with $NaBH_4/CeCl_3\cdot 7H_2O$ in methanol as described below (at Section 4.3.4) and obtained as crude **10** (1.54 g). The crude product **10** was dissolved in CH_2Cl_2, AcCl (20 mL) was added, and the resulting solution was stirred at room temperature for 12 h. The excess of unreacted acetyl chloride was evaporated (60 °C, 20 mmHg). The residue was chromatographed on a silica gel column eluting with EtOAc/hexane (1:4) to give triacetate **11** (0.85 g, 38%) and 1,4-diacetoxynaphthalene **12** (0.87 g, 49%).

4.3.2. Method B. The reduction of monoacetoxydiketone **9** (2.00 g, 9.09 mmol) was carried with $NaBH_4/CeCl_3\cdot 7H_2O$ in methanol as described below was obtained as crude **10** (1.96 g). The same procedure as described below (at Section 4.3.4) without $N_{2(g)}$ was applied for acetylation of **11**. After the removal of the solvent under reduced pressure (50 °C, 20 mmHg), the mixture was chromatographed on silica gel column with EtOAc/hexane (1:4) to give triacetate **11** (0.96 g, 42%) and 1,4-diacetoxynaphthalene **12** (1.02 g, 46%).

4.3.3. Method C. The reduction of monoacetoxydiketone **9** (1.86 g, 8.45 mmol) was carried with $NaBH_4/CeCl_3\cdot 7H_2O$ in methanol as described below (at Section 4.3.4) and obtained as crude **10** (1.78 g). Crude product **10** was dissolved in Ac_2O (30 mL) and to this

magnetically stirred solution was added anhydrous CH_3COONa (3.0 g, 4.9 mol). The reaction mixture was stirred at 90 °C for 12 h. The mixture was cooled to 0 °C and was added Et_2O (100 mL) and then a saturated solution of $NaHCO_3$. The mixture was extracted with Et_2O (2×100 mL) and H_2O (50 mL). The extracts were combined, dried over Na_2SO_4, and were evaporated (60 °C, 20 mmHg). The mixture was chromatographed on silica gel column with EtOAc/hexane (1:4) to give triacetate **11** (1.02 g, 39%) and 1,4-diacetoxynaphthalene **12** (0.80 g, 39%).

4.3.4. Method B under $N_{2(g)}$. Acetoxydiketone **9** (2.65 g, 12 mmol) was dissolved in methanol (50 mL) and then $CeCl_3 \cdot 7H_2O$ (8.93 g, 24 mmol) was added in two portions. The resulting solution was cooled to 0 °C by external application of an ice bath. Sodium borohydride ($NaBH_4$) (0.90 g, 24 mmol) was then added at such a rate that the temperature of the reaction mixture did not rise significantly above 0 °C. After the addition of $NaBH_4$ was complete, the external cold bath was removed, and the reaction mixture was allowed to warm gradually to ambient temperature with stirring for 2 h. The reaction was then quenched via addition of water (10 mL) and the resulting mixture was then extracted with ether (3×100 mL). The combined ether layers were washed successively with brine and then with water. The organic layers were combined, dried over Na_2SO_4, and filtered, and the filtrate was concentrated in vacuo to afford crude product **10** (1-acetoxy-5,8-dihydroxy-1,4,4a,5,8,8a-hexahydronaphthalene) (2.59 g). To a stirred solution of crude *cis*-diol acetate **10** in pyridine (10 mL) under nitrogen atmosphere at room temperature was added acetic anhydride Ac_2O (12 mL). The resulting mixture was stirred magnetically at room temperature for 12 h. The mixture was poured into ice-water and was added 80 mL of 4 M HCl solution and then extracted with ether (3×50 mL). The combined organic extracts were washed with $NaHCO_3$ solution (30 mL) and water (50 mL) and then dried over Na_2SO_4. Removal of the solvent under reduced pressure gave triacetate **11** (3.30 g, 89%). White crystals, mp 93–95 °C (from hexane/CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 5.79 (m, 1H, −CH=CH, H-7), 5.63 (m, 2H, −CH=CH, H-2 and H-3), 5.47 (d, J=10.3 Hz, 1H, H-6), 5.40 (m, 1H, −CH−OAc, H-5), 5.28 (m, 2H, −CH−OAc, H-1 and H-4), 2.58 (m, 1H, −CH, H-4a), 2.47 (m, 1H, −CH, H-8a), 2.14 (m, 1H, −CH, H-8 one of geminal protons), 2.00 (s, 3H, −OAc), 1.93 (s, 3H, −OAc), 1.87 (m, 1H, −CH, H-8 one of geminal protons), 1.86 (s, 3H, −OAc). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 171.0 (C=O), 170.4 (C=O), 170.2 (C=O), 129.8 (C=C), 129.7 (C=C), 126.3 (C=C), 125.9 (C=C), 71.6 (C−O), 70.7 (C−O), 63.0 (C−O), 35.6 (−CH), 32.3 (−CH), 22.5 (−CH_2), 21.5 (−CH_3), 21.1 (−CH_3), 21.0 (−CH_3). IR ($CHCl_3$, cm^{-1}): 3036, 2918, 1740, 1437, 1371, 1235, 1195, 1083, 1058, 1032, 947, 885. Anal. Calcd for $C_{16}H_{20}O_6$: C, 62.33; H, 6.54. Found: C, 62.39; H, 6.51.

*4.3.4.1. 1,4-Diacetoxynaphthalene **12**.* Mp 123–125 °C (from CH_2Cl_2/hexane) (lit.;[16] 128–129 °C). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.90 (AA′ part of AA′BB′system, 2H, aromatic), 7.55 (BB′ part of AA′BB′system, 2H, aromatic), 7.28 (s, 2H, aromatic), 2.44 (s, 6H, −CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 169.6 (×2, C=O), 144.6 (×2, C quaternary), 127.9 (×2, C quaternary), 127.2 (×2, C=C), 121.9 (×2, C=C), 117.9 (×2, C=C), 21.2 (×2, −CH_3). IR ($CHCl_3$, cm^{-1}): 3041, 2936, 1764, 1738, 1502, 1370, 1232, 1207, 1192, 1175, 1060, 1015, 907.

4.4. (±)-(1*R**,2*R**,3*R**,4a*R**,5*S**,8*R**,8a*S**)-1,2,3,4,4a,5,8,8a Octahydronaphthalene-1,2,3,5,8-pentayl pentaacetate (14)

A 100 mL two-necked, round-bottomed flask, equipped with a magnetic stirrer and a nitrogen inlet, was charged with 0.94 g (8.05 mmol) of NMO, water (2 mL), and acetone (10 mL). To this solution were added a catalytic amount of OsO_4 (ca. 10 mg, 0.08 mmol) and 2.48 g (8.25 mmol) of triacetate **11**. The resulting mixture was stirred vigorously under nitrogen at 0 °C. During the overnight stirring, the reaction mixture became homogeneous. After stirring for 12 h, $NaHSO_3$ (0.40 g) and 2 g of Florisil slurried in 2 mL of water were added, the slurry was stirred for 1 h, and the mixture was filtered through a short pad 3 g of Celite in a 60 mL sintered glass funnel. The Celite cake was washed with acetone (3×20 mL). The filtrates were combined and solvent was removed to give the crude *cis*-diol-triacetate **13** (2.40 g, 87%). The crude *cis*-diol-triacetate **13** was dissolved in pyridine (8 mL). To the magnetically stirred solution was Ac_2O (10 mL) added and stirred at room temperature for 12 h. The mixture was poured into ice-water (50 mL) and was added 4 M HCl solutions (30 mL) and extracted with ethyl acetate (3×100 mL). The combined organic extracts were washed with $NaHCO_3$ solution (50 mL) and water (50 mL) and then dried over Na_2SO_4 and concentrated in vacuo. The crude material was purified by column chromatography on silica gel by using EtOAc/hexane (4.5:5.5) to give (±)-(1*R**,2*R**,3*R**,4a*R**,5*S**,8*R**,8a*S**)-1,2,3,4,4a,5,8,8a octahydronaphthalene-1,2,3,5,8-pentayl pentaacetate **14** (2.91 g, 85%). Compound **14** was recrystallized from CH_2Cl_2/hexane (1:4) as white crystals, mp 138–141 °C. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 5.74 (dd, A part of AB system, J=10.0, 3.7 Hz, 1H, −CH=CH, H-6 or H-7), 5.73 (m, 1H, H-2), 5.67 (dd, B part of AB system, J=10.0, 3.7 Hz, 1H, −CH=CH, H-6 or H-7), 5.46 (m, 1H, −CH−O, H-3), 5.37 (m, 2H, −CH−O, H-5 and H-8), 5.21 (dd, J=10.6, 6.7 Hz, 1H, −CH−O, H-1), 2.80 (m, 1H, bridgehead-CH, H-8a), 2.70 (m, 1H, bridgehead-CH, H-4a), 2.15 (s, 3H, −CH_3), 2.07 (s, 3H, −CH_3), 2.06 (s, 3H, −CH_3), 1.97 (s, 3H, −CH_3), 1.96 (s, 3H, −CH_3), 1.80 (m, 2H, −CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 170.7 (C=O), 170.5 (C=O), 170.4 (C=O), 170.3 (C=O), 170.0 (C=O), 129.7 (C=C), 126.2 (C=C), 71.7 (C−O), 70.5 (C−O), 69.9 (C−O), 69.1 (C−O), 62.9 (C−O), 37.3 (−CH), 28.6 (−CH), 25.0 (−CH_2), 21.6 (−CH_3), 21.3 (−CH_3), 21.2 (−CH_3), 21.0 (−CH_3), 20.9 (−CH_3). IR ($CHCl_3$, cm^{-1}): 3020, 2944, 1733, 1435, 1372, 1235, 1096, 1047, 948, 921, 837, 736. Anal. Calcd for $C_{20}H_{26}O_{10}$: C, 56.33; H, 6.15. Found: C, 56.43; H, 6.18. EIMS (m/z, %): 426 (M^+, 0.5), 324.0 (7), 263.7 (5), 222.0 (5), 203.9 (10), 161.9 (44), 143.7 (100), 132.9 (19), 116.9 (11), 104.9 (8), 94.9 (7), 67.9 (6).

4.5. (±)-(1*R**,2*S**,3*R**,4*S**,4a*R**,5*R**,6*R**,7*R**,8a*R**)-Decahydronaphthalene-1,2,3,4,5,6,7-heptayl heptaacetate (16)

A 100 mL two-necked, round-bottomed flask, equipped with a magnetic stirrer and a nitrogen inlet, was charged with 0.47 g (4.03 mmol) of NMO, water (2 mL), and acetone (8 mL). To this solution were added a catalytic amount of OsO_4 (ca. 10 mg, 0.08 mmol) and 1.72 g (4.03 mmol) of pentaacetate **14**. The resulting mixture was stirred vigorously under nitrogen at 0 °C. During the overnight stirring, the reaction mixture became homogeneous. After stirring for 12 h, $NaHSO_3$ (0.30 g) and 2 g of Florisil slurried in 2 mL of water were added, the slurry was stirred for 1 h, and the mixture was filtered through a short pad 3 g of Celite in a 60 mL sintered glass funnel. The Celite cake was washed with acetone (3×20 mL). The filtrates were combined and solvent was removed to give the crude *cis*-diol-pentaacetate **15** (1.69 g, 91%). The crude *cis*-diol-pentaacetate **15** was dissolved in pyridine (6 mL). To the magnetically stirred solution was added Ac_2O (8 mL) and stirred at room temperature for 12 h. The mixture was poured into ice-water (50 mL) and was added 4 M HCl solutions (20 mL) and extracted with ethyl acetate (3×100 mL). The combined organic extracts were washed with $NaHCO_3$ solution (50 mL) and water (50 mL) and then dried over Na_2SO_4 and concentrated in vacuo. The crude material was purified by column chromatography on silica gel by using EtOAc/hexane (2:3) to give (±)-(1*R**,2*S**,3*R**,4*S**,4a*R**,5*R**,6*R**,7*R**,8a*R**)-decahydronaphthalene-1,2,3,4,5,6,7-heptayl heptaacetate **16** (1.96 g, 89%). Compound **16** was recrystallized from CH_2Cl_2/hexane (1:3) as white crystals, mp 169–171 °C. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 5.46 (m, 1H, −CH−O), 5.35 (m, 2H, −CH−O), 5.30 (m, 1H, −CH−O), 5.23 (m,

2H, −CH−O), 5.05 (t, J=3.3 Hz, 1H, −CH−O), 2.87 (m, 1H, bridgehead-CH, H-4a or H-8a), 2.81 (m, 1H, bridgehead-CH, H-4a or H-8a), 2.21 (s, 3H, $-CH_3$), 2.08 (s, 3H, $-CH_3$), 2.06 (s, 3H, $-CH_3$), 2.02 (s, 3H, $-CH_3$), 2.00 (s, 3H, $-CH_3$), 1.95 (s, 3H, $-CH_3$), 1.94 (s, 3H, $-CH_3$), 2.04 (m, 1H, $-CH_2$, H-8 or H-8′), 1.86 (m, 1H, $-CH_2$, H-8 or H-8′). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 170.5 (C=O), 170.4 (C=O), 170.3 (×2, C=O), 170.2 (C=O), 169.2 (C=O), 168.8 (C=O), 70.2 (C−O), 69.6 (C−O), 69.3 (C−O), 68.7 (C−O), 68.4 (×2, C−O), 67.0 (C−O), 35.8 (−CH), 31.9 (−CH), 26.2 ($-CH_2$), 21.5 ($-CH_3$), 21.2 ($-CH_3$), 21.1 (×2, $-CH_3$), 21.0 ($-CH_3$), 20.9 ($-CH_3$), 20.8 ($-CH_3$). IR ($CHCl_3$, cm^{-1}): 3062, 2958, 1748, 1434, 1372, 1224, 1110, 1089, 1055, 948, 796, 736. Anal. Calcd for $C_{24}H_{32}O_{14}$: C, 52.94; H, 5.92. Found: C, 52.92; H, 5.97. EIMS (m/z, %): 544 (M^+, 0.5), 399.0 (3), 381.9 (6), 340.0 (3), 321.9 (32), 261.9 (18), 219.9 (33), 201.8 (79), 159.7 (100), 131.9 (15), 121.0 (6), 102.9 (5), 90.9 (3).

4.6. (±)-(1*R**,2*S**,3*R**,4*S**,4a*R**,5*R**,6*R**,7*R**,8a*R**)-Decahydronaphthalene-1,2,3,4,5,6,7-heptaol (17)

Heptaacetate **16** (2.05 g, 3.76 mmol) was dissolved in 50 mL of absolute methanol. While dry NH_3 was being passed through the solution, the mixture was stirred at room temperature for 10 h. Evaporation of MeOH and formed acetamide gave (±)-(1*R**,2*S**,3*R**,4*S**,4a*R**,5*R**,6*R**,7*R**,8a*R**)-decahydronaphthalene-1,2,3,4,5,6,7-heptaol **17** in nearly quantitative yield (0.86 g, 92%), mp 250–253 °C (recrystallized from D_2O). ^{1}H NMR (400 MHz, D_2O, ppm) δ 3.99 (m, 3H, −CH−O), 3.78 (m, 3H, −CH−O), 3.67 (m, 1H, −CH−O), 2.21 (m, 1H, −CH), 2.10 (m, 1H, −CH), 1.60 (m, 2H, $-CH_2$). ^{13}C NMR (100 MHz, D_2O, ppm) δ 74.1 (−C−O), 72.7 (−C−O), 70.7 (−C−O), 69.9 (−C−O), 69.7 (−C−O), 69.4 (−C−O), 68.5 (−C−O), 38.9 (−CH), 34.2 (−CH), 27.7 ($-CH_2$). IR ($CHCl_3$, cm^{-1}): 3400, 2992, 2964, 2891, 1457, 1441, 1371, 1343, 1192, 1122, 1057, 1035, 1032, 962, 926. Anal. Calcd for $C_{10}H_{18}O_7$: C, 48.00; H, 7.25. Found: 48.13; H, 7.29.

4.7. X-ray structure determination

For the crystal structure determination, the single-crystals of compounds **14** and **16** were used for data collection on a four-circle Rigaku R-AXIS RAPID-S diffractometer (equipped with a two-dimensional area IP detector). The graphite-monochromatized Mo Kα radiation (λ=0.71073 Å) and oscillation scans technique with $\Delta\omega$=5° for one image were used for data collection. The lattice parameters were determined by the least-squares methods on the basis of all reflections with $F^2>2\sigma(F^2)$. Integration of the intensities, correction for Lorentz and polarization effects, and cell refinement were performed using Crystal Clear (Rigaku/MSC Inc., 2005) software.[24] The structures were solved by direct methods using SHELXS-97 and refined by a full-matrix least-squares procedure using the program SHELXL-97.[25] H atoms were positioned geometrically and refined using a riding model. The final difference Fourier maps showed no peaks of chemical significance. *Crystal data for* ***14***: $C_{20}H_{26}O_{10}$; crystal system, space group: orthorhombic, *Pbca*; (no: 61); unit cell dimensions: a=8.0876(2), b=15.2604(4), c=35.1662(7) Å, α=90°, β=90°, γ=90°; volume: 4340.21(18) $Å^3$; Z=8; calculated density: 1.305 g cm^{-3}; absorption coefficient: 0.105 mm^{-1}; F(000): 1808.0; θ range for data collection 2.3–30.7°; refinement method: full-matrix least-square on F^2; data/parameters: 2989/276; goodness-of-fit on F^2: 1.027; final R indices [$I>2\sigma(I)$]: R_1=0.0824, wR_2=0.229; R indices (all data): R_1=0.167, wR_2=0.261; largest diff. peak and hole: 586 and −0.332 e $Å^{-3}$; CCDC-914761. *Crystal data for* ***16***: $2(C_{24}H_{32}O_{14})\cdot CH_2Cl_2$. The asymmetric unit contains two heptaacetate **16** and one dichloromethane solvent molecule. For clarity, only one heptaacetate molecule was shown in the Fig. 2; crystal system, space group: monoclinic, $P2_1/c$; (no: 14); unit cell dimensions: a=8.5251(2), b=14.0722(5), c=49.4144(11) Å, α=90°, β=92.340(2)°, γ=90°; volume: 5923.2(3) $Å^3$; Z=4; calculated density: 1.316 g cm^{-3}; absorption coefficient: 0.194 mm^{-1}; F(000): 2472; θ range for data collection 2.2–31.0°; refinement method: full-matrix least-square on F^2; data/parameters: 6880/721; goodness-of-fit on F^2: 1.108; final R indices [$I>2\sigma(I)$]: R_1=0.0836, wR_2=0.1461; R indices (all data): R_1=0.216, wR_2=0.255; largest diff. peak and hole: 0.715 and −0.381 e $Å^{-3}$; CCDC-915426.

4.8. Investigation of inhibition effect on CA

In the present study compound **17** was investigated for its in vitro inhibition effect on CA I and II, which was purified from fresh blood according to literature procedure.[23] Carbonic anhydrase activity was assayed by following the change in absorbance at 348 nm of 4-nitrophenylacetate (NPA) to 4-nitrophenylate ion over a period of 3 min at 25 °C using a spectrophotometer (CHEBIOS UV–VIS) according to the method described by Verpoorte et al.[26] The enzymatic reaction, in a total volume of 3.0 mL, contained 1.4 mL of 0.05 M Tris–SO_4 buffer (pH 7.4), 1 mL of 3 mM 4-nitrophenylacetate, 0.5 mL of H_2O, and 0.1 mL of enzyme solution. A reference measurement was obtained by preparing the same cuvette without enzyme solution. The inhibitory effects of **17** were examined. All compounds were tested in triplicate at each concentration used. Different inhibitor concentrations were used. Control cuvette activity in the absence of inhibitor was taken as 100%. For each inhibitor, an Activity (%)–[Inhibitor] graph was drawn. To determine K_i values, three different inhibitor concentrations were tested. In these experiments, 4-nitrophenylacetate was used as substrate at five different concentrations (0.15–0.75 mM). The Lineweaver–Burk curves were drawn.[21]

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