SYNTHESIS OF OXAZOLIDINE ANALOGS OF BIOTIN AND EPIBIOTIN

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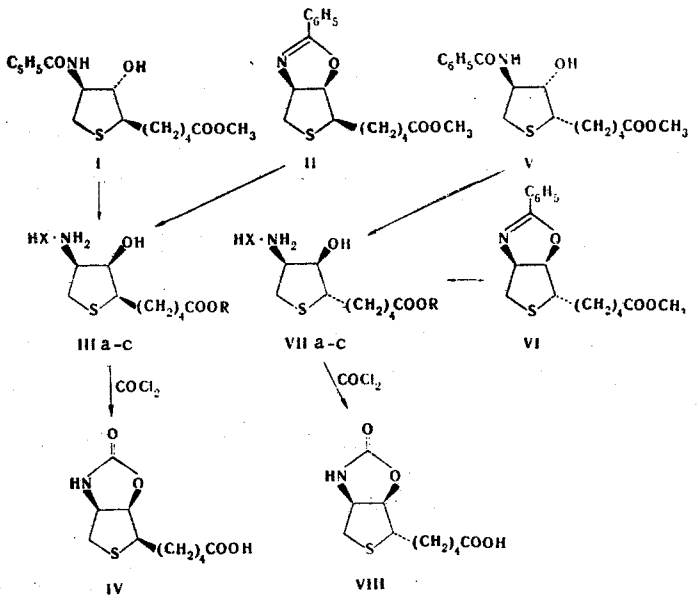
d*l*-cis- and d*l*-trans-6-(4-Carboxybutyl)-2-oxo-cis-hexahydrothieno[3,4-d]oxazoles were synthesized by the action of phosgene on r-4-amino-c-3-hydroxy-c (or, respectively, t)-2-(4-carboxybutyl)thiophan hydrohalides. It is shown that the acid hydrolysis of r-4-benzamido-t-3-hydroxy-c (or t)-2-(4-methoxycarbonylbutyl)thiophans is accompanied by inversion to give r-4-amino-c-3-hydroxy-c (or t)-2-(4-alkoxycarbonylbutyl)thiophans.

In order to study the effect on the biological activity of modification of the biotin – cis-tetrahydro-2-oxothieno[3,4-d]imidazoline-4-valeric acid – molecule, we synthesized oxazolidine analogs of biotin and epibiotin. One NH group, the hydrogen atom of which can be replaced by a carboxyl group is retained in these analogs, and the possibility of fulfillment of the principal biocatalytic function of biotin – carbon dioxide transfer in the biosyntheses of lipids, amino acids, carbohydrates, nucleic acids, and other metabolic reactions – is thereby retained in the modified molecule.

The biotin and epibiotin analogs d*l*-cis- (IV) and, respectively, d*l*-trans-6-(4-carboxybutyl)-2-oxo-cis-hexahydrothieno[3,4-d]oxazole (VIII) were synthesized by phosgenation of r-4-amino-c-3-hydroxy-c (respectively, t)-2-(4-methoxycarbonylbutyl or 4-carboxybutyl)thiophan hydrohalides (IIIa-c, VIIa-c).



III a X=Br, R=CH$_3$; b X=Cl, R=CH$_3$; c X=Cl, R=H; VII a X=Br, R=C$_2$H$_5$; b X=Cl, R=CH$_3$; c X=Cl, R=H

To obtain III and VII we used the previously observed [1] ability of trans-4-benzamido-3-hydroxythiophans to undergo inversion of configuration under conditions of acid hydrolysis, which leads to cis-4-amino-

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TABLE 1. Parameters of the PMR Spectra of Solutions of IIIa, IV, VII, VIIa, and VIII in Deuteropyridine (at 34°C)*

Compound	δ, ppm					J, Hz				
	2-H	3-H	4-H	5-H′	5-H″	$J_{2,3}$	$J_{3,4}$	$J_{4,5'}$	$J_{4,5''}$	$J_{5'5''}$
IIIa	3,50—3,80	5,07	4,41	3,55	3,55	3,4	3,4	$\Sigma J_{4,5}=18,0$		
VIIa	3,57—3,80	4,80	4,43	3,56	3,56	4,1	4,1	$\Sigma J_{4,5}=13,6$		
	3a-H	4-H′	4-H″	6-H	6a-H	$J_{3a,4'}$	$J_{3a,4''}$	$J_{4',4''}$	$J_{6,6a}$	$J_{3a,6a}$
IV	4,59	2,90	2,90	3,24	5,06	$\Sigma J_{3a,4}=4,2$			3,9	6,9
VIII	4,62	3,11	2,85	3,36	4,95	4,9	2,4	12,7	2,1	7,5

*The chemical shifts of the protons for IIIa, VIIa, and IV were determined as the centers of the corresponding multiplets. The corresponding AB systems were isolated and calculated in the determination of the 4-H′ and 4-H″ and 3a-H and 6a-H chemical shifts for VIII. The spin—spin coupling constants (SSCC) were determined as the distances between the resonance lines in the spectra.

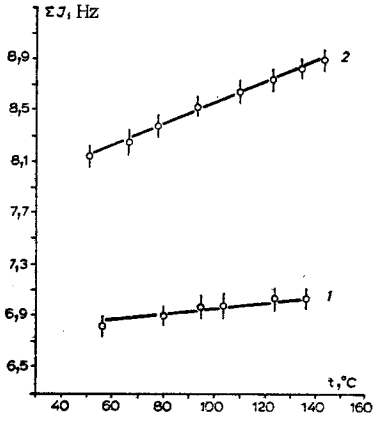

Fig. 1. Temperature dependences of the sum of $J_{2,3}$ and $J_{3,4}$ for solutions of IIIa (1) and VIIa (2) in deuteropyridine.

3-hydroxythiophans [1]. We showed that the hydrolysis of trisubstituted thiophans in refluxing hydrobromic acid also occurs with inversion of configuration and leads to the formation of hydrobromides IIIa and VIIa in 90 and 30% yields, respectively.

To establish the configuration of IIIa and VIIa we used hydrolysis of 2-phenyl-cis- (II) and 2-phenyl-trans-6-(4-methoxycarbonylbutyl)-cis-3a,4,6,6a-tetrahydrothieno[3,4-b]oxazoline (VI) in hydrobromic acid. In this case we isolated the same hydrobromides IIIa and VIIa, respectively. It is known that the configuration of the substituents in the thiophan ring is retained during opening of the oxazoline ring (for example, see [2-4]). Thus hydrohalides IIIa and VIIa, obtained from oxazolines II and VII, should retain their configuration, whereas the hydroxyl and amino groups should be found in the cis position. Consequently, IIIa is r-4-amino-c-3-hydroxy-c-2-(4-methoxycarbonylbutyl)thiophan hydrobromide, and IIIa is r-4-amino-c-3-hydroxy-t-2-(4-ethoxycarbonylbutyl)thiophan hydrobromide.

These structures for IIIa and VIIa were also confirmed by the PMR spectroscopic data (Table 1). The signal of the proton attached to C_3 in the spectra of these compounds is practically a triplet, and it therefore seemed possible to study the temperature dependence only of the sum of $J_{2,3}$ and $J_{3,4}$ (Fig. 1). As seen in Fig. 1, the sum of $J_{2,3}$ and $J_{3,4}$ for IIIa is practically independent of the temperature, whereas an appreciable temperature dependence (0.73 Hz as the temperature changes by 92.5°, Fig. 1) is observed for VIIa. This difference in the behavior of the sums of the vicinal spin—spin coupling constants (SSCC) as the temperature changes is in conformity with the previously proposed model of the temperature dependences of cis- and trans-vicinal constants [5]. In fact, the protons attached to C_2, C_3, and C_4 in IIIa are cis-oriented to one another, i.e., the determined sum of the constants is the sum of the cis-vicinal constants and therefore should be practically independent of the temperature. Another possible explanation

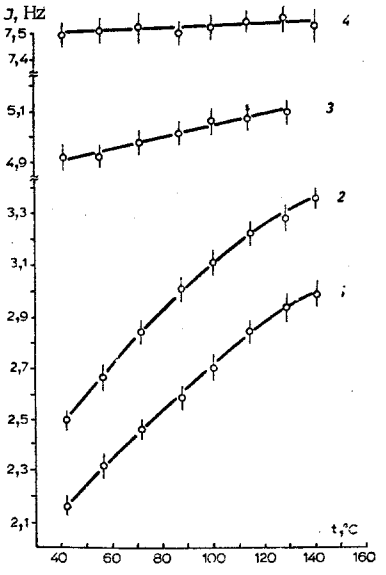

Fig. 2

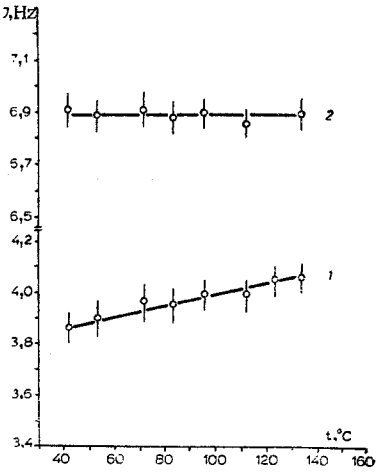

Fig. 3

Fig. 2. Temperature dependences of $J_{6,6a}$ (1), $J_{3a,4}$ (2), $J_{3a,4}$ (3), and $J_{3a,6a}$ (4) for a solution of VIII in deuteropyridine.

Fig. 3. Temperature dependences of $J_{6,6a}$ (1) and $J_{3a,6a}$ (2) for a solution of IV in deuteropyridine.

for the observed absence of a temperature dependence for IIIa is the zero difference in the energies of the conformers corresponding to the minima of the potential curve of pseudorotation [5]. In this case the trans-vicinal constants also should not depend on the temperature. Unfortunately, we were unable to follow the temperature dependence of the single trans-vicinal constant ($J_{4,5}$") for IIIa, inasmuch as the chemical shifts of the protons attached to C_5 in the spectra of this compound are close to one another over the investigated temperature range. However, the vicinal SSCC of IIIa are almost identical to the analogous constants of r-4-benzamido-c-3-hydroxy-c-2-(4-methoxycarbonylbutyl)thiophan [4], for which a considerable temperature dependence of $J_{4,5}$" has been traced [5], during which the sum of $J_{2,3}$ and $J_{3,4}$ remained practically unchanged. The closeness of the vicinal constants means that these two compounds are characterized by the same conformational state. Consequently, the results of the temperature experiments for IIIa are associated with a cis orientation of the protons attached to C_2, C_3, and C_4.

In the case of VIIa, the trans-vicinal SSCC ($J_{2,3}$), which, according to the model, should change as the temperature changes [and this is observed experimentally (Fig. 1)], enters into the measured sum of the constants.

Hydrochlorides IIIb and VIIb were isolated when oxazolines II and VI were refluxed in 7N hydrochloric acid, whereas acid hydrochlorides IIIc and VIIc were isolated when oxazolines III and VII were refluxed in 2.5 N sodium hydroxide solution (after acidification of the reaction mixtures with HCl).

The parameters of the PMR spectra of solutions of IV and VIII in deuteropyridine are presented in Table 1. To determine the type of fusion of the thiophan and oxazolidine rings and the configuration of the substituent attached to C_6 in these compounds we analyzed the PMR parameter and measured the temperature dependences of the vicinal SSCC constants (Figs. 2 and 3).

In the case of VIII the magnitudes of the two vicinal constants are practically independent of the temperature ($J_{3a,4}$', $J_{3a,6a}$, Fig. 2, curves 3 and 4), whereas a substantial temperature dependence ($\Delta J_{6,6a}$ = 0.83 Hz and $\Delta J_{3a,4}$" = 0.85 Hz as the temperature increases by 99.5°, Fig. 2, curves 1 and 2) is observed for the other two constants ($J_{6,6a}$ and $J_{3a,4}$"), and this makes it possible to assign the latter to trans-vicinal constants. In this case $J_{6,6a}$ is found among the trans constants, and this unambiguously indicates the trans configuration of the substituent attached to C_6 with respect to the oxazolidine ring. The low $J_{6,6a}$ value (2.1 Hz at 34°, Table 1) also confirms the trans orientation of the substituent attached to C_6, inasmuch as the unusually large (for a five-membered ring) torsion angle (60-66°) should correspond to such a low value of the cis-vicinal constant.

TABLE 2. Calculated (for Conformations A and B) and Experimental (for IV and VIII) Vicinal Spin–Spin Coupling Constants Along the 6–6a and 3a–4 Bonds*

Conformation	$\psi_{6,6a(3a,4)}$, °	Calc., $J_{6,6a}$, Hz		Expt., $J_{6,6a}$, Hz		Calc., $J_{3a,4'}$, Hz	Calc., $J_{3a,4''}$, Hz	Calc., $\Sigma J_{3a,4}$, Hz	Expt., $J_{3a,4'}$, Hz (VIII)	Expt., $J_{3a,4''}$, Hz (VIII)	Expt., $\Sigma J_{3a,4}$, Hz (IV)
		cis	trans	cis (IV)	trans (VIII)						
A	20	6,5—10,5	0,0—0,5	3,9	2,1	6,5—10,5	0—0,5	6,5—11,0	4,9	2,4	4,2
	30	6,0—9,5	0			6,0—9,5	0	6,0—9,5			
	40	4,5—7,0	0			4,5—7,0	0	4,5—7,0			
B	20	6,5—10,5	6,0—9,0			6,5—10,5	6,0—9,0	12,5—19,5			
	30	6,0—9,5	7,0—11,0			6,0—9,5	7,0—11,0	13,0—20,5			
	40	4,5—7,0	8,5—13,0			4,5—7,0	8,5—13,0	13,0—20,0			

*In the calculation of the theoretical values of the constants the torsion angle along the 6–6a (3a–4) bond was varied from 20 to 40°. The ranges of the calculated vicinal constants are given in the table; and this corresponds to variation of the angular dependence of J^0 from 8 to 12 Hz in the equations ($J^{180} = 1.35\ J^0$) [2].

In the case of IV the $J_{6,6a}$ value is practically independent of the temperature (Fig. 3, curve 1). The closeness of the vicinal constants of this compound and 2-phenyl-cis-6-(4-methoxycarbonylbutyl)-cis-3a,4,6,6a-tetrahydrothieno[3,4-d]oxazole (II), for which we were able to observe an appreciable temperature dependence of the single trans-vicinal constant ($J_{3a,4}$) [2] makes it possible to assert that the practical invariance of the $J_{6,6a}$ value with temperature is explained by cis orientation of the corresponding protons rather than by identical energies of the conformers corresponding to the minima of the potential energy curve for this compound. The increase (by a factor of almost two) in $J_{6,6a}$ as compared with VIII (3.9 and 2.1 Hz, respectively, Table 1) also constitutes evidence in favor of a cis orientation of the substituent attached to C_6. Thus IV is cis-6-carboxybutyl-cis-3a,4,6,6a-tetrahydrothieno[3,4-d]oxazolidone.

It is known that the configuration of substituents does not change upon phosgenation [1, 6]. The identical character of the configurations of the substituents in IV and III and VIII and VII once again confirms the correctness of the configurational assignments made in this paper.

The conformational state and the type of fusion of the rings for IV and VIII were determined, as in [3], by means of the angular dependence of the vicinal SSCC [7]. It was found that cis fusion of the thiophan and oxazolidine rings and the following conformational equilibrium are characteristic for these compounds:

IV $R' = (CH_2)_4COOH$, $R'' = H$; VIII $R' = H$, $R'' = (CH_2)_4COOH$

A comparison of the trans-vicinal constants calculated for conformations A and B along the 6–6a and 3a–4 bonds with the experimentally observed values (Table 2) showed that conformation A is the primary conformation for IV and VIII. The same preferred conformation was found for biotin [8] and for other bicyclo[3.3.0]octane analogs that we previously investigated in [2-4].

EXPERIMENTAL METHOD

The PMR spectra of the compounds were recorded with an Hitachi R-20A spectrometer (60 MHz) with tetramethylsilane as the internal standard. The assignment of the signals to definite protons was confirmed by the ratio of the integral intensities of the signals and double resonance experiments. In the measurement of the temperature dependences the temperature was determined prior to and after recording of the spectrum from the temperature dependence of the chemical shifts of the protons of ethylene glycol (the ethylene glycol sample was subjected to prior calibration). Traces of oxygen were removed from the samples by blowing an inert gas through them. The vicinal constants were averaged with respect to 12 to 20 measurements to raise the accuracy.

r-4-Amino-c-3-hydroxy-c-2-(4-methoxycarbonylbutyl)thiophan Hydrobromide (IIIa). A) A solution of 1.5 g (5 mmole) of I [4] in 20 ml of hydrobromic acid was refluxed for 7 h, after which it was cooled and

extracted with benzene. The extract was concentrated, 20 ml of methanol was added to the concentrated extract, and the mixture was treated with activated charcoal and concentrated to 3-4 ml. The concentrated solution was allowed to stand at 0-3° for 16 h, and the resulting precipitate was separated to give 1.26 g (90%) of colorless crystals with mp 159-159.5° (from methanol). Found: C 37.9; H 6.5; Br 26.0%. $C_9H_{17}NO_3S \cdot HBr$. Calculated: C 38.2; H 6.4; Br 25.5%.

B) Under conditions similar to those in method A, 0.74 g (90%) of IIIa, with mp 159-159.5° (from methanol), was obtained from 1 g (3 mmole) of 2-phenyl-cis-6-(4-methoxycarbonylbutyl)-cis-3a,4,6,6a-tetrahydrothieno[3,4-d]oxazoline (II) hydrochloride [2]. No melting-point depression was observed for a mixture of this product with the compound obtained by method A.

r-4-Amino-c-3-hydroxy-c-2-(4-methoxycarbonylbutyl)thiophan Hydrochloride (IIIb). A solution of 0.7 g (2 mmole) of II in 12 ml of a 5 N hydrochloric acid was refluxed for 5 h, after which it was cooled and extracted with benzene. The extract was allowed to stand at 0-3° for 12-16 h, and the resulting precipitate was separated to give 0.5 g (90%) of colorless plates with mp 208-209° (from methanol). Found: C 44.4; H 7.31; Cl 13.6%. $C_{10}H_{19}NO_3S \cdot HCl$. Calculated: C 44.5; H 7.5; Cl 13.2%.

r-4-Amino-c-3-hydroxy-c-2-(4-carboxybutyl)thiophan Hydrochloride (IIIc). A) A 0.7-g (2 mmole) sample of II was added to 20 ml of a 2.5 N solution of sodium hydroxide, and the mixture was refluxed for 3 h. It was then cooled, acidified with 5 N hydrochloric acid, and extracted with benzene. The extract was concentrated to dryness, and the residue was extracted with methanol. The alcohol extracts were concentrated to 3-4 ml, and the concentrated solution was allowed to stand at 0-3° for 16-18h. The resulting precipitate was separated to give 0.4 g (72%) of colorless plates with mp 152-153° (from methanol). Found: C 42.4; H 7.37; Cl 14.3%. $C_9H_{12}NO_3S \cdot HCl$. Calculated: C 42.3; H 7.1; Cl 13.9%.

B) A 1 N solution of sodium hydroxide was added to 1 g (3 mmole) of IIIb, and the mixture was refluxed for 30 min. It was then acidified to pH 1-2 with 5 N hydrochloric acid and concentrated to dryness. The residue was extracted with methanol, the extracts were concentrated to 3-4 ml, and the concentrated solution was allowed to stand at 0-3°. The resulting precipitate was separated to give 0.9 g (94%) of a product with mp 152-153°. No melting-point depression was observed for a mixture of this product with the compound obtained by method A.

r-4-Amino-c-3-hydroxy-t-2(4-ethoxycarbonylbutyl)thiophan Hydrobromide (VIIa). A) A solution of 3 g (10 mmole) of V [4] in 40 ml of concentrated hydrobromic acid was refluxed for 9 h, after which it was cooled and extracted with benzene. The extract was concentrated, 40 ml of alcohol was added to the residue, and the mixture was treated with activated charcoal and concentrated to 3-4 ml. The concentrated solution was allowed to stand at 0-3°, and the resulting precipitate was separated to give 0.87 g (30%) of colorless prisms with mp 146-147° (from alcohol). Found: C 39.8; H 6.7; Br 24.2%. $C_{11}H_{21}NO_3S$. Calculated: C 40.2; H 6.7; Br 24.1%.

B) Under conditions similar to those in method A, 1.74 g (90%) of VIIa, with mp 146-147° (from alcohol), we obtained from 2 g (10 mmole) of 2-phenyl-c-6-(4-methoxycarbonylbutyl)-cis-3a,4,6,6a-tetrahydrothieno[3,4-d]oxazoline (VI) [4]. No melting-point depression was observed for a mixture of this product with the compound obtained by method A.

r-4-Amino-c-3-hydroxy-t-2-(4-methoxycarbonylbutyl)thiophan Hydrochloride (VIIb). The method used to synthesize IIIb was used to obtain 0.8 g (95%) of colorless needles with mp 173-174° (from methanol) from 1 g (3 mmole) of VI. Found: C 44.0; H 7.2; Cl 13.5%. $C_{10}H_{19}NO_3S \cdot HCl$. Calculated: C 44.5; H 7.5; Cl 13.2%.

r-4-Amino-c-3-hydroxy-t-2-(4-carboxybutyl)thiophan Hydrochloride (VIIc). Under conditions similar to those in the preparation of II (method B), 0.45 g (80%) of colorless plates with mp 151-152° (from alcohol) was obtained from 0.7 g of VI. Found: C 42.8; H 7.0; Cl 13.7%. $C_9H_{17}NO_3S \cdot HCl$. Calculated: C 42.3; H 7.1; Cl 13.9%.

dl-cis-6-(4-Carboxybutyl)-2-oxo-cis-hexahydrothieno[3,4-d]oxazole (IV). Xylene (30 ml) and 60 ml of 8% aqueous sodium carbonate solution were added at 0° to a solution of 1 g (3 mmole) of IIIa (or IIIb, c) in 2 ml of 2.5 N sodium hydroxide solution, and phosgene was bubbled through the solution up to pH 1-2. The xylene solution was separated, and the aqueous solution was extracted with chloroform. The xylene solution and chloroform extracts were combined and concentrated, a 2.5% alcohol solution of sodium hydroxide was added to the residue, and the mixture was refluxed for 30 min. It was then concentrated and extracted with methanol saturated with hydrogen chloride. The extract was concentrated to 2-3 ml and

the concentrated solution was allowed to stand at 0-3° for 16 h. The resulting precipitate was removed by filtration to give 0.72 g (87%) of colorless needles with mp 195-196° (from methanol) and R_f 0.85 [elution with an n-butyl alcohol–amyl alcohol–water–acetic acid system (20 : 20 : 12 : 1) and development with a 0.2% solution of potassium permanganate]. Found: 49.1; H 5.9; S 13.0%. $C_{10}H_{15}NO_4S$. Calculated: C 49.0; H 6.2; S 13.1%.

dl-trans-6-(4-Carboxybutyl)-2-oxo-cis-hexahydrothieno[3,4-d]oxazole (VIII). Under conditions similar to those in the preparation of IV, 0.75 g (90%) of colorless needles with mp 180-181° (from methanol) and R_f 0.83 (in the same system as in the case of IV) was obtained from 1 g (3 mmole) of VIIa (or VIIb, c). Found: C 49.2; H 6.3; S 12.6%. $C_{10}H_{15}NO_4S$. Calculated: C 49.0; H 6.2; S 13.1%.

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MASS SPECTRA OF 4-ACYLAMINO-3-HYDROXY(OR ACYLOXY)THIOPHANS

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The mass spectra of cis- and trans-4-acylamino-3-hydroxy(or acyloxy)thiophans were investigated. The general principles of fragmentation under the influence of electron impact were established. A difference in the intensities of the peaks of the fragments formed at an ionizing-electron energy of 14 eV was observed for some of the cis and trans isomers of 3,4-substituted thiophans.

Up to now, only individual mass-spectrometric studies of thiophans were known; for example, unsubstituted thiophans [1] and α-alkylthiophans [2] have been studied. In connection with our investigation of the stereochemistry of di- and trisubstituted thiophans [3-6], it was of interest to study the fragmentation of cis- and trans-4-acylamino-3-hydroxy(or acyloxy)thiophans. A total of 25 thiophan derivatives, of which 11 were cis- and trans-isomeric pairs, were studied.

As a result of the study we established the general principles for I-XIV and the effect of the type of substituent on the fragmentation process. The relative intensities of the ion peaks of hydroxyaminothiophans are presented in Table 1.

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