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On the Methanolysis of KBBL, an Immunostimulant Derived from Ascorbic Acid

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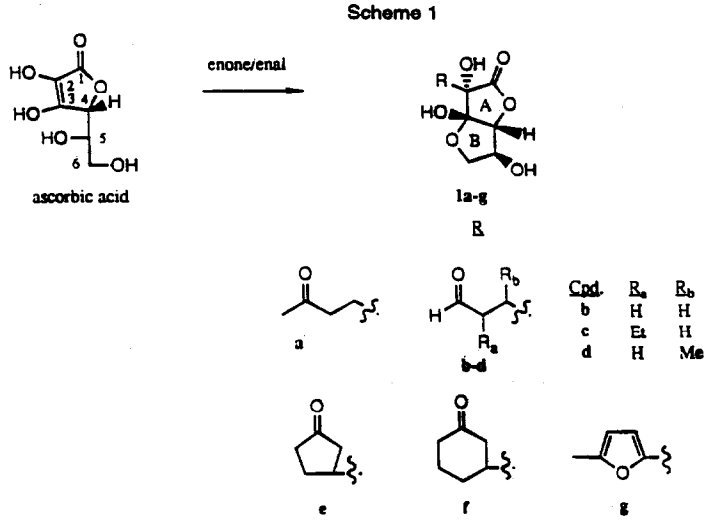
Abstract: Treatment of the immunostimulant KBBL (**1a**) in dry methanol with Amberlyst® 15 as catalyst yielded the epimeric spiro ketals **4a** and **4b** along with a minor amount of the pyranoid ketal **2**. Contrary to a literature report, treatment of KBBL in 2% methanolic HCl gave the rearranged methyl ester **3** rather than ketal **2**. Ketals **2**, **4a**, and **4b** are potentially useful intermediates for the further elaboration of the ascorbic acid nucleus of KBBL because the ketone and one hydroxyl of the butyrolactone nucleus (derived from ascorbic acid) are simultaneously protected as an internal ketal. X-Ray structures of these compounds were obtained, contributing to our knowledge of the structure of the KBBL family of immunostimulants.

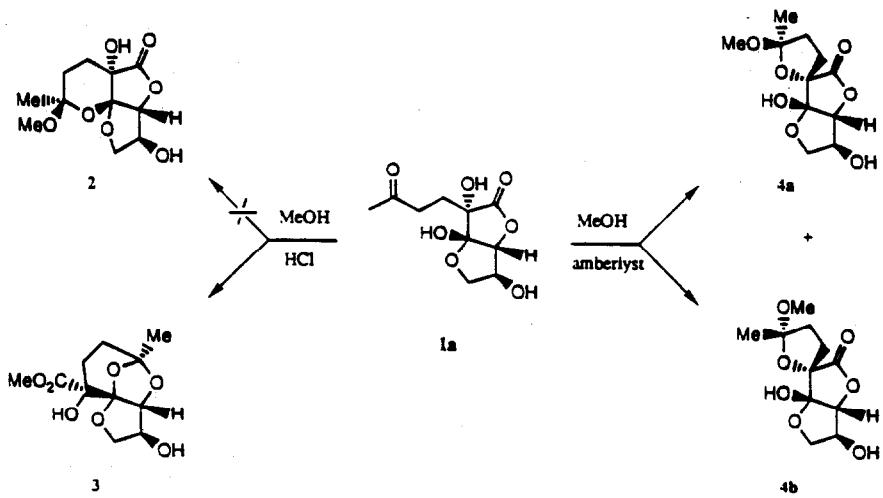
Progress in the derivatization of ascorbic acid has contributed to our understanding of the chemistry, biosynthesis, catabolism, and biological role *in vivo* of this important substance.[1] A new series of C2 alkylated ascorbic acid derivatives, butyrolactones **1a-g**, was synthesized by Fodor *et al.* by the condensation of ascorbic acid with α,β-unsaturated aldehydes and ketones.[2a-d] These compounds exist as hemiketals in solution and in the solid state. The formation of C2 alkylated ascorbic acid derivatives by this reaction is noteworthy because alkylation of the mono-anion of ascorbic acid by alkyl halides is complicated by competing O3 alkylation.[1] Eger *et al.* have also studied the equilibrium between the various acetal forms of the acrolein adduct **1b**[3a-c], and have prepared the related derivatives **1c** and **1d**.[3b] Butyrolactones **1a-g** are of biological interest,[2d] since KBBL (ketobutylbutyrolactone, **1a**) and MFBL (methylfurylbutyrolactone, **1g**) possess T cell-dependent immunostimulatory activity[4a-i] and the ability to increase the survival rates of immunocompromised tumor-bearing animals.[4j]

Scheme 1



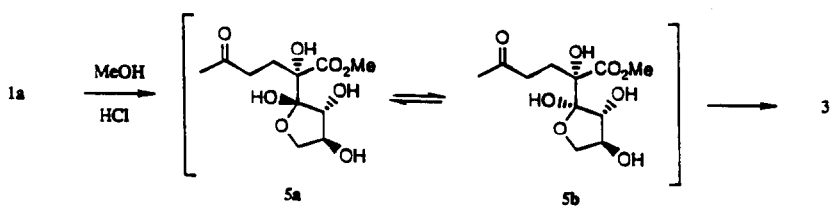
In our efforts to further derivatize C2 alkylated ascorbic acid derivatives such as KBBL (**1a**), we considered various protecting group strategies and were drawn to a report that internal ketal **2** was obtained by treatment of KBBL in methanolic HCl (Scheme 2).[2a] Ketal **2** was viewed as an attractive intermediate for the modification of KBBL, especially the ascorbic acid nucleus, because the side chain ketone carbonyl and anomeric tertiary hydroxyl are simultaneously protected and can be unmasked under mild acidic conditions. We have reinvestigated the acid catalyzed reaction of KBBL with methanol and now wish to report that the product obtained under the conditions above is not ketal **2**, but rather the rearranged methyl ester **3**, and that reaction under milder conditions yields primarily the epimeric spiro ketals **4a** and **4b**.

Scheme 2



Methanolysis of KBBL was performed in 2% methanolic HCl, as described by Fodor *et al.*, using a modified purification procedure involving flash chromatography instead of direct recrystallization. A crystalline material was obtained in 30% yield which had a similar melting point, 155-157°C (lit.[2a] 154-156°C), and a virtually identical ^{13}C NMR spectrum[2a,5] to that reported for **2**. Since the previous structural assignment of **2** was not definitive and was based on analogy of its ^{13}C NMR and ^{1}H NMR spectra to that of the related derivative **6c** (see Scheme 4),[2a] an X-ray structure was obtained, revealing that it was, surprisingly, the rearranged methyl ester **3** (Figure 1). The formation of **3** presumably occurs by a pathway involving methanolysis of the lactone ring to produce tetrahydroxy keto ester **5a**, epimerization of the anomeric hydroxyl to give **5b**, followed by internal ketalization at the proximal vicinal *cis* diol unit of the furan ring (Scheme 3).

Scheme 3



To see if it was indeed possible to generate ketal **2**, milder conditions were examined. Thus, a solution of KBBL in methanol was treated with a suspension of dry Amberlyst® 15 for 3 days, at which time two major products not corresponding to ester **3** were observed by TLC. These compounds were isolated by flash chromatography and were identified as the epimeric spiro ketals **4a** and **4b**. Interestingly, a third minor product which eluted between **4a** and **4b** was also isolated (in 1% yield) and proved to be the elusive pyranoid ketal **2**. The structures of **2**, **4a**, and **4b** were tentatively assigned based on their ^{13}C and ^{1}H NMR spectra and were ultimately established by X-ray crystallography (Figure 1). The formation of **2**, **4a**, and **4b** under these conditions appears to occur under thermodynamic control, as resubjection of either **2** or **4a** to the reaction conditions led to a product ratio similar to that observed in the original reaction, as judged by TLC. The combined chromatographed yield of ketals **4a**, **4b**, and **2** was modest (45%) and suffered from incomplete consumption of KBBL. It was later found that the reaction could be driven to completion and the reaction time shortened to 16 h by the addition of activated 3Å molecular sieves. During chromatography, however, some minor hydrolysis to regenerate KBBL could not be avoided. The ketals were best stored under cold anhydrous conditions, as prolonged storage under ambient conditions resulted in hydrolysis.

Two general structural observations may be made based on the X-ray structures of **2**, **4a**, **4b**, and those reported for the furan derivative **1g**[2b] and the hemiketals **6a**[3a] and **6c**[2a] derived from the acrolein-ascorbic acid adduct **1b** (Scheme 4). First, compounds **1g**, **4a**, **4b**, **6a**, and **6c**, which are capable of undergoing equilibration at the anomeric hydroxyl, have a *cis* A-B ring fusion. This is expected based on the known relative stability (6 kcal/mole) of *cis* 5-5 ring systems over the corresponding trans systems.[6] Also, compound **2**, in which the A-B ring system is locked, has a *cis* ring fusion. Second, the anomeric effect is seen in the structures of the related tricyclic pyrano-fused ketal **2** and the hemiketal **6c**, where the anomeric β-methoxy and the β-hydroxyl groups, respectively, are in the axial positions in the pyran ring.

The solution ^{13}C NMR spectra (DMSO-d_6) of ketals **2**, **4a**, and **4b** are listed in Table 1 along with, for comparison, the solid state ^{13}C NMR spectra of acetals **6a** and **6c**, as reported by Eger *et al.*[3c] In the case of epimers **4a** and **4b**, corresponding chemical shifts were within 1 ppm with the exception of the two anomeric centers (C_C and C_i). Similar chemical shifts were also observed between the pairs **2**:**6c** and **4a**:**6a**, with the major difference being the expected downfield shift of the anomeric carbons (C_i) of **2** and **4a** bearing the additional methyl group.

In conclusion, we have shown that reaction of the immunostimulant KBBL (**1a**) with methanol under mild acidic conditions yields the epimeric spiro ketals **4a** and **4b** as the major products. A small amount of the pyranoid ketal **2** is also formed. The product ratios reflect thermodynamic control. Under more forcing conditions, methanolysis of the lactone ring and subsequent internal ketalization occurs to produce methyl ester **3**. Ketals **2**, **4a**, and **4b** are potentially useful intermediates for the further elaboration of KBBL, since the side chain ketone carbonyl and one of the hydroxyls are simultaneously protected. The X-ray structures of **2**, **4a**, and **4b**, along with those of related butyrolactone analogs reported in the literature, contribute to our understanding of the structure of this emerging class of immunostimulants.

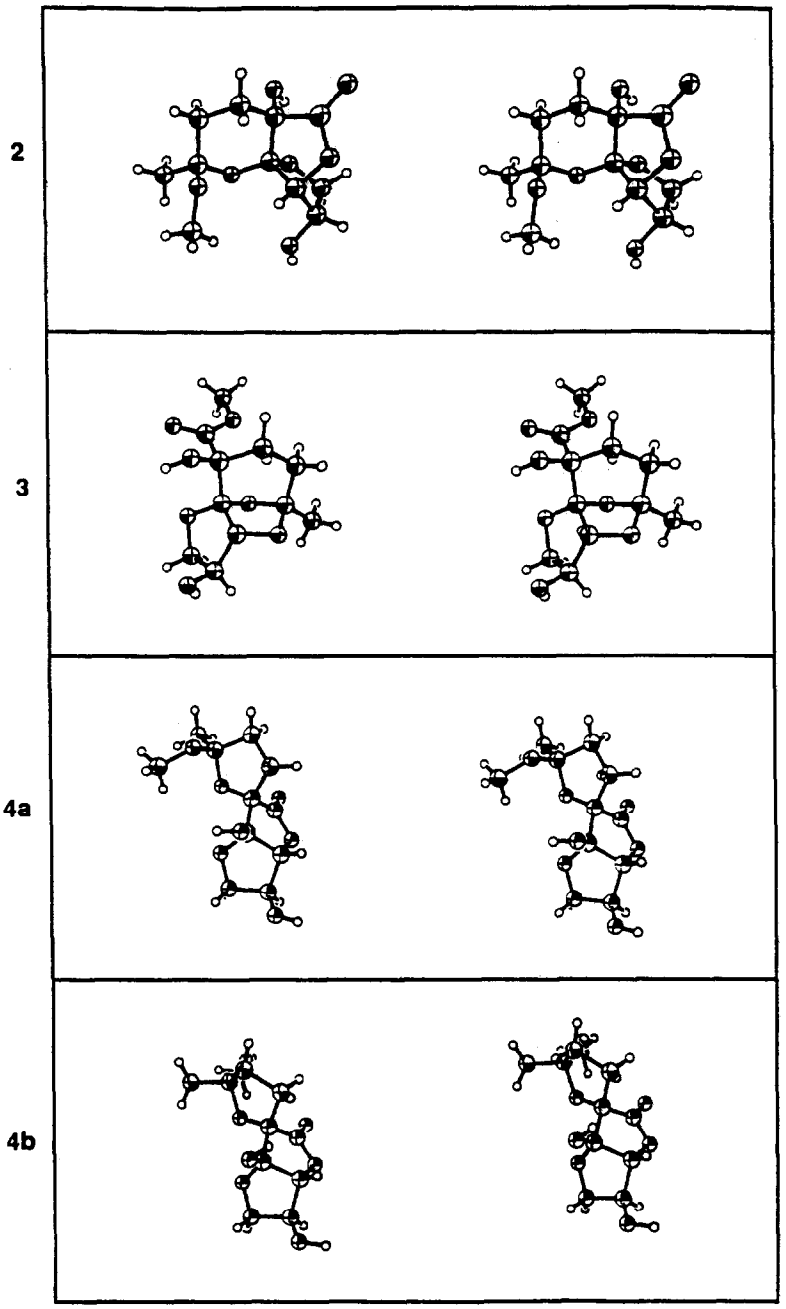
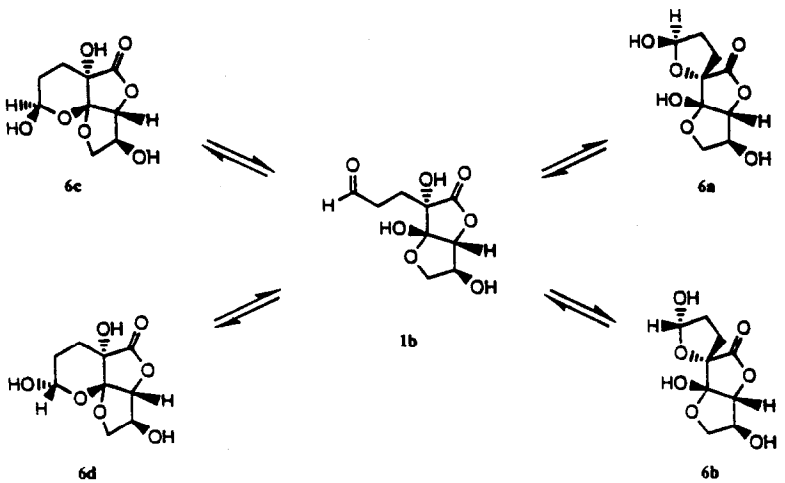


Figure 1. ORTEP stereo representation of the X-ray structures of **2**, **3**, **4a** and **4b**.

Scheme 4



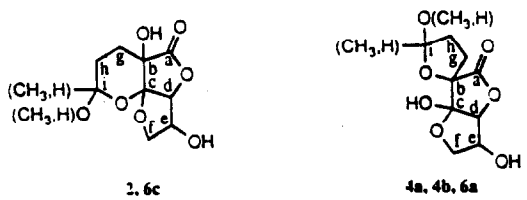


Table I. ^{13}C NMR Chemical Shifts of **2**, **4a** and **4b** in Solution and **6a** and **6c** in the Solid State

Atom Index	Solution (DMSO)			Solid State[3c]	
	2	**4a**	**4b**	**6a**	**6c**
a	175.5	174.9	175.0	175.2	174.0
b	69.7	87.7	87.3	88.0	72.0
c	107.4	109.5	106.9	107.5	107.5
d	85.9	86.0	86.0	85.3	86.1
e	75.3	75.0	75.0	78.5	78.5
f	73.1	73.6	73.7	73.1	74.7
g	27.1	30.5	31.1	28.4	29.5
h	28.3	35.7	36.7	32.3	29.5
i	99.7	105.9	109.8	101.2	94.9
OCH_3	48.4	48.7	48.6		
CH_3	24.0	22.6	22.6		

EXPERIMENTAL SECTION

^1H and ^{13}C NMR spectra were determined on a Bruker AM 300 spectrometer at 300 MHz and 75 MHz, respectively. Chemical shifts are expressed in ppm relative to deuterio dimethyl sulfoxide or deuterium oxide. Significant ^1H NMR signals are tabulated in order (number of protons, multiplicity, and coupling constant (Hz)). Infrared (IR) spectra were determined on a Perkin-Elmer 283B. Elemental analyses were performed by the Pfizer Analytical Chemistry Department or the Schwarzkopf Microanalytical Laboratory. Melting points are uncorrected and were obtained in open capillaries on an Electrothermal digital melting point apparatus.

KBBL was prepared according to the procedure of Fodor *et al.*[2a] Amberlyst® 15(wet) ion exchange resin and anhydrous methanol packaged in a Sure/Seal® bottle were purchased from Aldrich Chemical Company.

Flash chromatography was performed using Silica Woelem (32-63 μm) or 'Baker' Silica Gel (40 μm). Analytical thin-layer chromatography (TLC) was performed on Merck Kieselgel 60 F_{254} using phosphomolybdic acid for visualization.

[3S-(3α, 3aα, 5β, 8α, 8aβ)]-Hexahydro-3,8-dihydroxy-5-methyl-2H-5,8a-epoxyfuro[3,2-b]oxepin-8-carboxylic acid Methyl Ester (3)

Treatment of 5.00 g of KBBL in methanolic HCl according to the literature procedure[2a] yielded 6.6 g of a semi-solid after silver carbonate neutralization, drying, and evaporation. The residue was boiled in 200 ml of EtOAc and the undissolved solids were removed by filtration. The EtOAc layer was evaporated and the residue was triturated in ether/hexane to give 3.2 g of a yellow solid. Purification by flash chromatography using 90 g of silica gel and a 3:1 EtOAc:hexane eluant afforded 1.60 g (30%) of **3** after ether trituration, mp 155-157°C (lit.[2a] 154-156°C). ^{13}C NMR (D_2O) δ 25.4, 33.3, 37.2, 56.0, 75.3, 77.6, 79.6, 88.7, 114.9, 117.0, 175.9. IR ($CHCl_3$) 3530 and 1733 cm-1

[3'S-[3'α(S*),3'aβ,6'β,6'aβ]]-Hexahydro-3'a,6'-dihydroxy-5-methoxy-5-methyl-spiro[furan-2(3H),3'(2'H)-furo[3,2-b]furan]-2'-one (4a), [3'S-[3'α(R*),3'aβ,6'β,6'aβ]]-Hexahydro-3'a,6'-dihydroxy-5-methoxy-5-methyl-spiro[furan-2(3H),3'(2'H)-furo[3,2-b]furan]-2'-one (4b), and [3S-(3α,3aα,5aβ,8α,9aS*)]-3,5a-dihydroxy-8-methoxy-8-methyl-hexahydro-2H,5H-furo[3',2':2,3]furo[3,4-b]pyran-5-one (2).

To a solution of 5.00 g of KBBL in 30 ml of dry methanol was added 1 g of pre-dried Amberlyst® 15, prepared by washing Amberlyst® 15 (wet) ion exchange resin with methanol and then costripping with isopropanol and methanol in that order, and the mixture was stirred at RT for 3 days under N_2. The catalyst was removed by filtration and the filtrate was concentrated to an oil, which was purified by flash chromatography (150 g of silica gel) using 2-3% MeOH in $CHCl_3$ as eluant and collecting 35 ml fractions. Fractions 39-43 yielded 860 mg (16%) of a white foam, which was crystallized from ether to provide 603 mg (11%) of **4a** as a white solid, mp 123-126°C. The analytical sample was prepared by recrystallization from 4:1 hexane-EtOAc, mp 123-125 °C. R_f=0.48, 9:1 $CHCl_3$-MeOH; ^1H NMR (DMSO-d_6) δ1.45 (s, 3H), 1.87-1.94 (2H, m), 2.02-2.08 (1H, m), 2.45-2.56 (1H, m), 3.18 (3H, s), 3.89 (1H, dd, J = 4 and 9), 4.18 (1H, dd, J = 6 and 9), 4.26-4.30 (1H, m), 4.44 (1H, s), 5.63 (1H, bd s), 6.62 (1H, bd s); ^{13}C NMR (DMSO-d_6) d 22.6, 30.5, 35.7, 48.7, 73.6, 75.0, 86.0, 87.7, 105.9, 109.5, 174.9; IR ($CHCl_3$) 3550, 3380 and 1800 cm-1. Anal. Calcd for $C_{11}H_{16}O_7$: C, 50.77; H, 6.20. Found: C, 50.73; H, 6.37.

Fractions 58-70 yielded 1.48 g (28%) of a white foam which was crystallized from ether to provide 811 mg (15%) of **4b** as a white solid, mp 117-119°C. The analytical sample was prepared by recrystallization from 4:1 hexane-EtOAc, mp 117-118 °C. R_f=0.41, 9:1 $CHCl_3$-MeOH; ^1H NMR (DMSO-d_6) δ1.43 (3H, s), 1.91-2.02 (3H, m), 2.30-2.45

(1H, m), 3.25 (3H, s), 3.86 (1H, dd, J = 4 and 9), 4.18-4.33 (2H, m), 4.46 (1H, s), 5.61 (1H, bd s), 6.81 (1H, bd s); ^{13}C NMR (DMSO-d_6) δ 22.6, 31.1, 36.7, 48.6, 73.7, 75.0, 86.0, 87.3, 106.9, 109.8, 175.0. Anal. Calcd for $C_{11}H_{16}O_7$: C, 50.77; H, 6.20. Found: C, 50.93; H, 6. 30.

Fraction 52 was evaporated to give 85 mg (1.6%) of a white foam which was crystallized from ether-hexane to provide 20 mg (0.38%) of **2**, mp 168-170°C. R_f=0.45, 9:1 $CHCl_3$-MeOH; ^1H NMR (DMSO-d_6) δ1.33 (3H, s), 1.64-2.05 (4H, m), 3.26 (3H, s), 3.89 (1H, dd, J = 3 and 9), 4.16 (1H, dd, J = 6 and 9), 4.32-4.36 (1H, m), 4.63 (s, 1H), 5.71 (1H, J = 4), 5.96 (1H, s); ^{13}C NMR (DMSO-d_6) δ 24.0, 27.1, 28.3, 48.4, 69.7, 73.1, 75.3, 85.9, 99.7, 107.4, 175.5; IR ($CHCl_3$) 3550 and 1800 cm-1. Anal. Calcd for $C_{11}H_{16}O_7$: C, 50.77; H, 6.20. Found: C, 50.48; H, 6. 25.

Single Crystal X-Ray Analysis. A representative crystal was surveyed and a 1 Å data set (maximum sin θ/λ = 0.5) was collected on a Siemens R3RA/v diffractometer. Atomic scattering factors were taken from the International Tables for X-ray Crystallography.[7] All crystallographic calculations were facilitated by the SHELXTL[8] system. All diffractometer data were collected at room temperature. Pertinent crystal, data collection, and refinement parameters are summarized in Table II.

A trial structure was obtained by direct methods. This trial structure refined routinely. Hydrogen positions were calculated wherever possible. The methyl hydrogens and the hydrogens on oxygen were located by difference Fourier techniques. The hydrogen parameters were added to the structure factor calculations but were not refined. The shifts calculated in the final cycle of least squares refinement were all less than 0.1 of their corresponding standard deviations. The final R-index was 3.62%. A final difference Fourier revealed no missing or misplaced electron density.

The refined structure was plotted using the SHELXTL plotting package. The absolute configuration was not determined in this analysis. Coordinates, anisotropic temperature factors, distances and angles were submitted to this Journal for deposition to the Cambridge Crystallographic Data Centre.

Table II. Crystal and Refinement Parameters of the X-ray Structures of Compounds **2**, **3**, **4a** and **4b**

	Compound			
A. Crystal Parameters	**2**	**3**	**4a**	**4b**
formula	$C_{11}H_{16}O_7$ (260.3)	$C_{11}H_{16}O_7$ (260.3)	$C_{11}H_{16}O_7$ (260.3)	$C_{11}H_{16}O_7$ (260.3)
crystallization medium	ethyl acetate			ethyl acetate
crystal size, mm	0.10 x 0.32 x 0.62	0.06 x 0.31 x 0.64	0.25 x 0.30 x 0.68	0.34 x 0.36 x 0.37
cell dimensions	a = 5.948 (2)Å	a = 13.635 (5) Å	a = 8.281 (3) Å	a = 6.557 (1) Å
	b = 11.148 (2) Å	b = 7.507 (2) Å	b = 11.449 (5) Å	b = 9.979 (2) Å
	c = 17.551 (3) Å	c = 12.122 (4) Å	c = 13.044 (4) Å	c = 18.488 (6) Å
	α = 90.0°	α = 90.00°	α = 90.00°	α = 90.00°
	β = 90.0°	β = 93.70 (3)°	β = 90.00°	β = 90.00°
	γ = 90.0°	γ = 90.00°	γ = 90.00°	γ = 90.00°
	V = 1163.7 (3) $Å^3$	V = 1238.2 (7) $Å^3$	V = 1236.7 (7) $Å^3$	V = 1209.7 (5) $Å^3$
space group	$P2_12_12_1$	C2	$P2_12_12_1$	$P2_12_12_1$
molecules/unit cell	4	4	4	4
density calcd, g/cm^3	1.485	1.40	1.40	1.43
linear absorption factor	1.075 mm^{-1}	9.65 cm^{-1}	9.66 cm^{-1}	9.88 cm^{-1}
B. Refinement Parameters				
number of reflections	729	705	768	756
nonzero reflections (I >3.0σ)	699	691	729	735
R-index[9]	3.62%	0.041	0.030	0.061
GOF[10]	1.44	1.13	1.36	1.18
scale factor	–	1.235 (4)	1.302 (3)	1.254 (8)
secondary extinction factor[11] χ	43(4) x 10^{-3}	NONE	7 (1) x 10^{-3}	5 (1) x 10^{-2}

References and Notes

†To whom correspondence should be sent concerning the X-ray structural determinations of **2**, **3**, **4a**, and **4b**.

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(5) A consistent 2 ppm upfield shift of all resonances relative to those reported for **2** in ref. 2a was observed. *p*-Dioxane, used as an internal standard, displayed a resonance at 69.5 ppm.

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(9) R-index = Σ||Fo|-|Fc||/Σ|Fo|

(10) GOF = $[\Sigma w(Fo^2-Fc^2)^2/(m-s)]^{1/2}$ where $w = [\sigma^2(F) + |g|F^2|]^{-1}$ g = 0.0010

(11) $F^* = F[1+0.002\,\chi\,F^2/\sin(2\,\theta)]^{-1/4}$

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