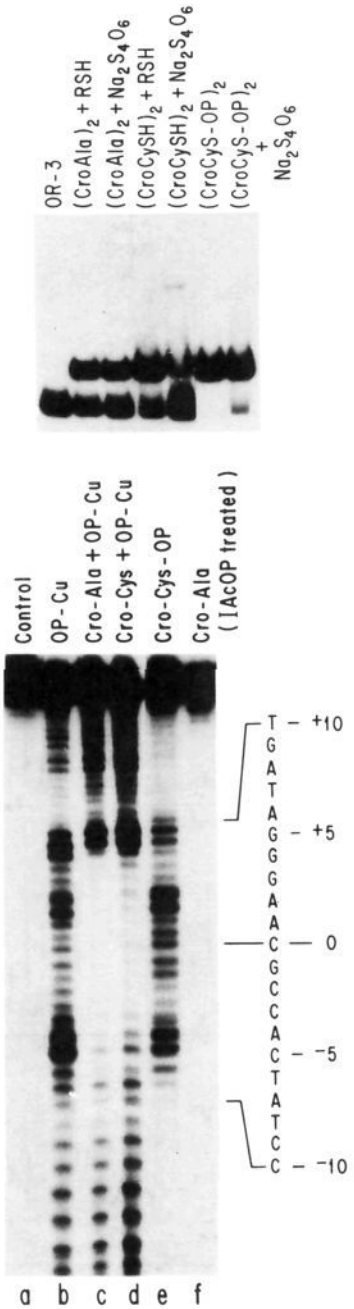

Figure 2. Top (A): Binding of Cro variants to OR-3 analyzed by gel retardation. Cro derivatives and OR-3 double-stranded DNA were incubated in a buffer composed of 10 mM Tris·HCl, pH 7.9, 1.0 mM Na_2EDTA, 50 mM KCl, 0.1 mg of BSA/mL, 10% glycerol in 15–30 μL. Cro derivatives were pretreated with either 2-mercaptoethanol (1–3 μL of 1/150 2-mercaptoethanol) or sodium tetrathionate (1–3 μL of 40 mM) for 30 min on ice (as indicated) prior to addition of nucleic acids (2 μL of 2 mg pdIdC-pdIdC/mL; 0.1 mL) (5′-^{32}P-labeled -OR-3-, ca. 3.0–12.0 × 10^5 cpm) and incubated at ambient temperature for 20 min. Except for lane designated OR-3, all other lanes also contain specific complexes formed with Cro variants and prebinding treatments as indicated above each gel lane. Bottom (B): Scission of nontemplate (upper) strand of OR-3. Lane a: probe alone. All other lanes are scission patterns of reactions carried out within the acrylamide matrix following separation of Cro-OR-3 complex by gel retardation (see Figure 2A). Footprinting reactions proceeded for 5 min; A66C Cro-Cys-OP mediated reactions, *for 10 min. All reactions were quenched by addition of 2,9-dimethyl*-1,10-phenanthroline to ca. 1.4 mM.[14] DNA fragments were recovered and analyzed as previously described.

crystallographic[6] and NMR[7] studies. Because of its ability to direct functional groups to the minor groove without sacrificing binding affinity, A66C Cro-Cys may be useful for comparing the efficiency of different DNA scission reagents. Isolation of A66C Cro-Cys variants with high affinity to different recognition sequences may provide a family of nucleolytic agents valuable in the analysis of chromosomal DNAs.

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Registry No. DNA nuclease, 9003-98-9; copper, 7440-50-8; 3-mercaptopropionic acid, 107-96-0.

High-Resolution Electrospray Mass Spectra of Large Molecules

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Impressive ionization methods for molecules of greater than 10 000 molecular weight (MW) include plasma desorption,[1] fast atom bombardment,[1] matrix-assisted laser desorption,[1] and electrospray ionization (ESI).[2,3] ESI, effective even with bovine albumin dimer (MW 133 000), has the unique advantage of producing multicharged molecular ions whose peaks are thus in the much more convenient mass/charge region of m/z 500–2000. For an ESI spectrum containing relatively few masses, m values can be derived from the larger number of m/z values because each z must be an integer value. As an alternative applicable to even complex spectra containing many masses, such as those of dissociated primary ions from tandem mass spectrometry (MS/MS, MS^n), resolution sufficient to separate the $^{13}C/^{12}C$ isotopic peaks will also define their charge state because the peak mass separation must be 1.0034 Da (daltons).[3] For instrumentation employed to date for ESI[2,3] (as well as other methods),[1] unit resolution has not been achieved for MW > 10 000, although mass-measuring accuracy of ~0.01% has been reported for samples up to MW 40 000 despite resolving powers (RP) of ~1000.[2,3] Using Fourier transform mass spectrometry (FTMS)[4] with ESI[3] of femtomole samples, we now report greatly improved RP and mass accuracy with fast broadband recording of all ions of m/z >400.

For each spectrum,[3] ions are introduced (through five pumping stages) during 7 ms (~3×10^{-15} mol of sample expended; front trapping plate 0 V, back 8 V) and allowed to cool for 1000 s (both *trapping plates 8 V), the ions are excited (sweep −100 Hz* μs^{-1}; both trapping plates 1 V), and the broadband signal is received (bandwidth 75 kHz, 1.7-s scan). Figure 1A clearly shows eight

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Table I. Mass-Measuring Accuracy with External and Internal Calibration for Figure 2 Data

		cytochrome *c*		myoglobin		
	measd	calcd	ext calibr[a]	calcd	int calibr[b]	
RP	m/z[a]	m/z	$\Delta m/z$	m/z	$\Delta m/z$	Δm
34 000	942.004	942.024	*−0.020*	942.174	−0.150	−2.70
42 000	942.155	942.179	*−0.024*	942.285	−0.109	−1.97
56 000	942.238	942.256	*−0.018*	942.341	−0.082	−1.49
29 000	942.317	942.333	*−0.016*	942.397	−0.060	−1.08
34 000	942.382	942.410	*−0.028*	942.452	−0.050	−0.90
52 000	942.551	942.564	*−0.013*	942.564	*0.007*	*0.13*
38 000	942.602	942.642	−0.040	942.620	*0.002*	*0.04*
53 000	942.656	942.719	−0.063	942.675	*0.001*	*0.02*
46 000	942.715	942.796	−0.081	942.731	*0.004*	*0.07*
27 000	942.763	942.873	−0.110	942.787	*−0.003*	*−0.06*
23 000	942.832	942.950	−0.118	942.843	*0.009*	*0.16*
31 000	942.886	943.028	−0.142	942.898	*0.008*	*0.14*
25 000	942.945			942.954	*0.010*	*0.19*
61 000	942.995			943.010	*0.005*	*0.09*
21 000	943.064			943.065	*0.019*	*0.34*
		mean:[c]	*−0.020*		*0.006*	*0.11*
		σ:	*0.005*		*0.006*	*0.10*

[a] Using gramicidin S, MW 1141, as an external m/z standard. [b] Using cytochrome *c* as an internal m/z standard. [c] Mean of italic figures only.

peaks per 0.5 m/z units, establishing charge state 16+. The ~80-fold resolution improvement over previous ESI/FTMS spectra[3b] is due mainly to the thermal cooling of ions.[5-7] Single-scan spectra of equine cytochrome *c* taken sequentially at pressures of 3, 7, 8, 30, and 100 × 10^{-9} Torr gave RP values of 61K, 62K, 61K, 45K, and 33K, respectively.[8] Use of longer data sets improved RP at low pressure much more than at 10^{-7} Torr, indicating that increased pressure shortens the transient signal lifetime.

For the spectrum of a mixture of porcine and chicken cytochrome *c*, ΔMW = 7 Da (Figure 1), m/z scale calibration with gramicidin S, MW 1141, gave mass values (average of 14+ to 17+) of 12 288.71 ± 0.07 (error −0.52) and 12 236.02 ± 0.48 (error −0.20), respectively, for the most abundant isotopic peaks (MW_i). For an unknown, however, it is also necessary to identify the most abundant isotope, for which the abundance reproducibility[4] (possibly ≤10 ions per peak in Figure 1A) is a problem in matching abundances to those expected (Figure 1D). Although averaging 23 scans (Figure 1C) lowers the resolution with our present data system, this improves the abundance match, as does averaging these data for the 14+ to 17+ ions (Figure 1D);[9] remeasuring the same ions[6] should also help. Although the presence of a second component (Figure 1, part A vs part B) is obvious, at this signal level determining ΔMW = 7 is less certain;[9] better abundance accuracy will be more critical for distinguishing isotopically labeled biomolecules (e.g., replacement of H by D).[10]

The multiplicity of charge states provides an unusual opportunity for internal mass calibration. In Figure 2, isotopic peaks at m/z 942.564 (Table I) arise from both $(M + 13H)^{13+}$ of chicken cytochrome *c* and $(M + 18H)^{18+}$ of equine myoglobin. With the MW 1141 external standard, the average mass accuracies are −0.26 and −0.25 Da, respectively, while the m/z spacings clearly establish the presence of both 13+ and 18+ charge states. Use of cytochrome *c* as in *internal* m/z standard reduces the myoglobin mass error to +0.11 Da. Even higher mass accuracy is indicated by the overlapping isotopic peak (Figure 2, arrow,

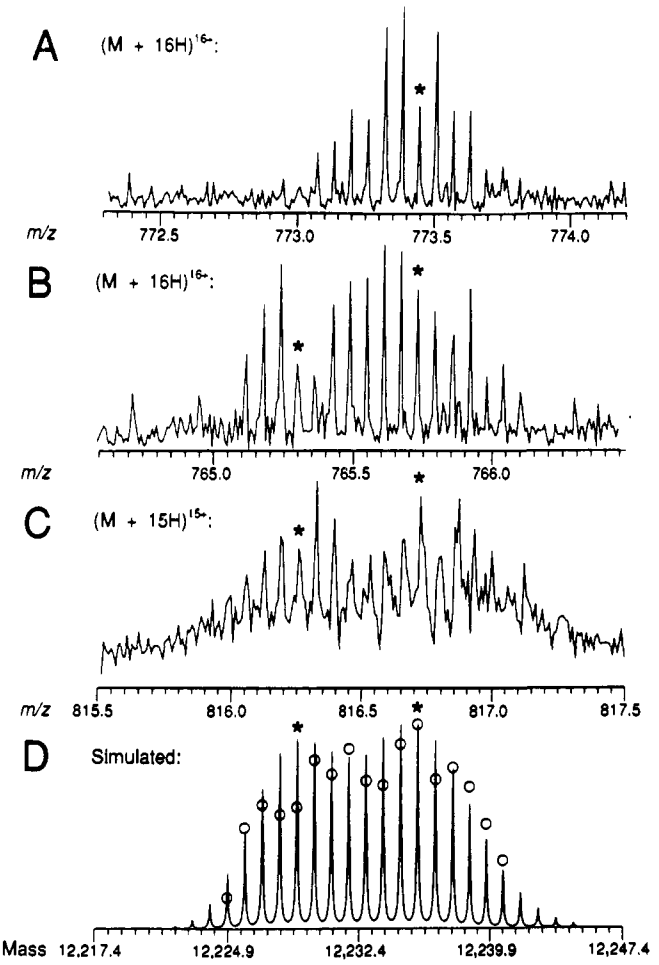

Figure 1. ESI/FT mass spectra of (A) equine cytochrome *c* (MW_i 12 358.34), single scan, average RP = 78K, and (B–D) 1:1 mixture of porcine (MW_i 12 229.23) and chicken (MW_i 12 236.22) cytochrome *c*; (B) single scan, average RP = 64K, and (C) 23 scans, average RP = 20K; (D) theoretical isotopic distribution. Asterisk: for each component, the isotopic peak calculated to be the most abundant. Circles: abundance averages from 23 scan spectra of 14+ for 17+. No adduct or fragment ions of m/z >400 were observed elsewhere in the spectra.

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(8) For albumin (MW 66 000), only RP 300 has been achieved to date (coherent transient lifetime <50 ms).

(9) M. W. Senko here has computed χ^2 values for calculated vs observed isotopic abundances; for a 10-scan spectrum of equine cytochrome *c* for errors of −1, 0, and +1 Da, values are 0.330, 0.048, and 0.123, respectively, while for the Figure 1D data for ΔMW = 6, for errors −1, 0, and +1, χ^2 = 0.898, 0.218, and 0.060; for ΔMW = 7 (*correct value*), for errors −1, 0, and +1, χ^2 = 0.042, *0.031*, and 0.116; for ΔMW = 8, for errors of −1, 0, and +1, χ^2 = 0.033, 0.061, and 0.141.

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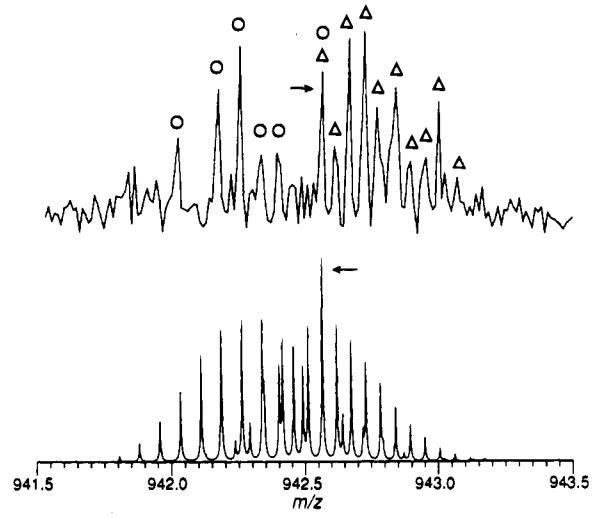

Figure 2. ESI/FT mass spectrum of a 1:1 mixture of (O) chicken cytochrome *c*, $(M + 13H)^{13+}$, and (Δ) equine myoglobin, $(M + 18H)^{18+}$. Top: measured spectrum, data in Table I. Bottom: calculated isotopic distribution. Arrow: *m/z* 942.564.

containing ~1:2 of the components) that still shows RP = 52K, a half-height width of *m* = 0.33 for *z* = 18; if this overlapping peak were shifted by 0.1 Da from the other, both peaks would have to be recorded at RP = 70K to produce the observed 52K. Note that this approaches the accuracy needed to distinguish between different elemental compositions; for example, replacing S by CH_6N changes the mass by 0.078 Da. The utility of this capability for unknown identification will be reported separately.

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Registry No. Cytochrome *c*, 9007-43-6.

Practical Asymmetric Synthesis of Both Erythro and Threo Aldols: Unusual Effect of Silyl Groups

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Despite innumerable investigations of asymmetric aldol methodologies in recent years,[1] little is known of the asymmetric synthesis of parent aldols, i.e., β-hydroxy aldehydes **1** (X = H) because of the great difficulty of generating chiral aldehyde enolates (or their equivalents) for aldol condensations and the instability of the resulting β-hydroxy aldehydes **1** (X = H).[2] The β-hydroxy aldehyde unit is undoubtedly a valuable synthetic intermediate for further carbon-chain elongation leading to 1,3-dihydroxy functionality, which is a fundamental structural unit

Scheme I

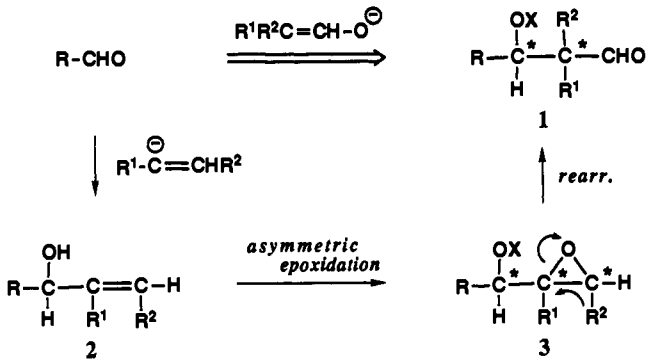

Table I. Effect of Silyl Groups on the Rearrangement of Erythro Epoxy Silyl Ether **4** with MABR[a]

entry	substrate **4**	yield,[b] % (ratio of **8**:**9**)[c]
1	R = *i*-Pr	78 (1:2.7)
2	R_3 = $PhMe_2$	65 (1.5:1)
3	R_3 = Ph_2Me	77 (6:1)
4	R_3 = *t*-$BuPh_2$	72 (10:1)
5	R = Ph	73 (40:1)

[a] Epoxide rearrangement was effected in CH_2Cl_2 with 2 equiv of MABR at −78 to −20 °C. [b] Isolated yield. [c] The threo:erythro ratios were determined by 200-MHz ^1H NMR or HPLC analysis.

embedded in numerous natural products of acetate and propionate origin.[3] In this context, we have studied a new, asymmetric synthesis of erythro and threo aldols based on the Lewis acid promoted rearrangement of optically active epoxy silyl ethers **3** (X = SiR_3),[4-6] which is readily derivable by Sharpless asymmetric epoxidation[7] of allylic alcohols **2** followed by simple silylation as illustrated in Scheme I. Since both erythro and threo epoxy silyl ethers are easily accessible in optically active forms,[8,9] the only remaining problem is the stereoselectivity of the epoxide rearrangement. Reported herein are our results, which successfully permit the practical asymmetric synthesis of both erythro and threo β-hydroxy aldehyde derivatives.

Rearrangement of erythro epoxy silyl ether **4** (R_3 = *t*-$BuMe_2$; >98% ee, $[\alpha]^{22}_D$ −6.80° (*c* 1.00, $CHCl_3$))[8] with exceptionally bulky, oxygenophilic methylaluminum bis(4-bromo-2,6-di-*tert*-butylphenoxide) (MABR), which has been recently developed in our laboratory as a highly effective epoxide-rearrangement agent,[4] yielded a mixture of threo and erythro β-siloxy aldehydes **8** and **9** (R_3 = *t*-$BuMe_2$) in 75% yield, though the observed threo/erythro selectivity was quite disappointing (ratio, ~1:1.4). Even bulky triisopropylsilyl ether **4** (R = *i*-Pr) showed poor selectivity (**8**:**9** (R = *i*-Pr) = 1:2.7). In marked contrast, however, erythro epoxy triphenylsilyl ether **4** (R = Ph; >98% ee, $[\alpha]^{23}_D$ +10.0° (*c* 1.02,

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(8) The optically active erythro epoxy alcohols are derived by Sharpless asymmetric epoxidation of allylic alcohols with Ti(O-*i*-Pr)$_4$/(+)-DIPT and *t*-BuOOH according to ref 7.

(9) The optically active threo epoxy alcohols can be prepared by the Mitsunobu inversion of the hydroxy group of optically active erythro isomers: Mitsunobu, O. *Synthesis* **1981**, 1.

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