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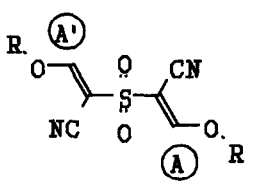
THE SYNTHESIS OF A POTENTIAL CROSS-LINKING REAGENT: 2,2'-SULFONYLBIS[3-BENZYLAMINO-(*E,E*)-*N*-(2-OXOETHYL)PROPENAMIDE]

Craig M. Bertha and Ramachandra S. Hosmane*

Laboratory for Chemical Dynamics, Department of Chemistry and Biochemistry
University of Maryland Baltimore County
Baltimore, Maryland 21228

The synthesis of the title bis-aldehydic reagent (3) with potential applications in biomacromolecular cross-linking is reported.

We have recently reported the synthesis, reactions, and applications of a few bifunctional organic reagents (BORs)[1a-d] as part of our broad program to explore novel organic reagents for potential biomedical applications. These BORs, as exemplified by reagents **1** and **2**, contained either bis(enol-ether)[1a,b,d] or bis(enamine)[1c] functionalities as the sites of cross-linking with proteins and/or nucleic acids. Both are highly electrophilic reagents and operate by initial conjugate addition of amine



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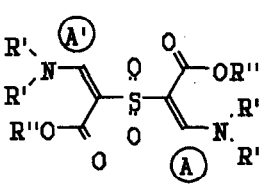

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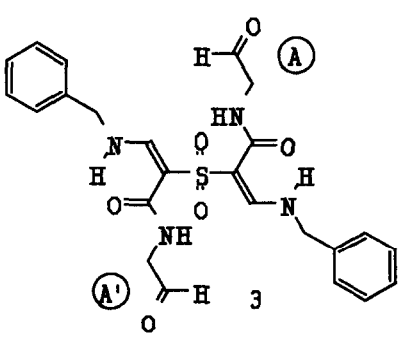


3

nucleophiles to their respective cross-linking sites (A,A'), followed by elimination of either alcohol or dialkylamine, producing stable secondary enamines as products. We have also demonstrated[1b,d] the versatility and high reactivity of **1** toward the building blocks of both proteins (amino acids) and nucleic acids (nucleosides). In addition, we have shown the reagent's utility in cross-linking deoxy- and oxyhemoglobins.[1a] Likewise, the reagent **2** was shown to undergo facile amine exchange reactions with a variety of primary amines.[1c] Nevertheless, the two reagents suffer from two major drawbacks: one, their cross-linking tethers (the distance A-A'), as revealed by single-crystal X-ray diffraction analyses of **1**[2] and **2**,[1c] have a short span of only 5-6 Å, limiting their practical utility. For example, while the tether

of **1** is long enough to cross-link the two dissimilar subunits of hemoglobin (α_1 to β_1 or α_2 to β_2), it falls short of cross-linking the two like subunits (α_1 to α_2 or β_1 to β_2, see below), an essential characteristic sought in a cross-linker for preparation of modified hemoglobins to be used as blood substitutes.[3,4] The second drawback of these reagents is their practically rigid configurational (*E,E*) and conformational (*anti*) stereochemistry at the cross-linking sites at ambient temperatures, leaving little flexibilty for proper alignment of the bridging arms for optimal interactions with biomacromolecules.

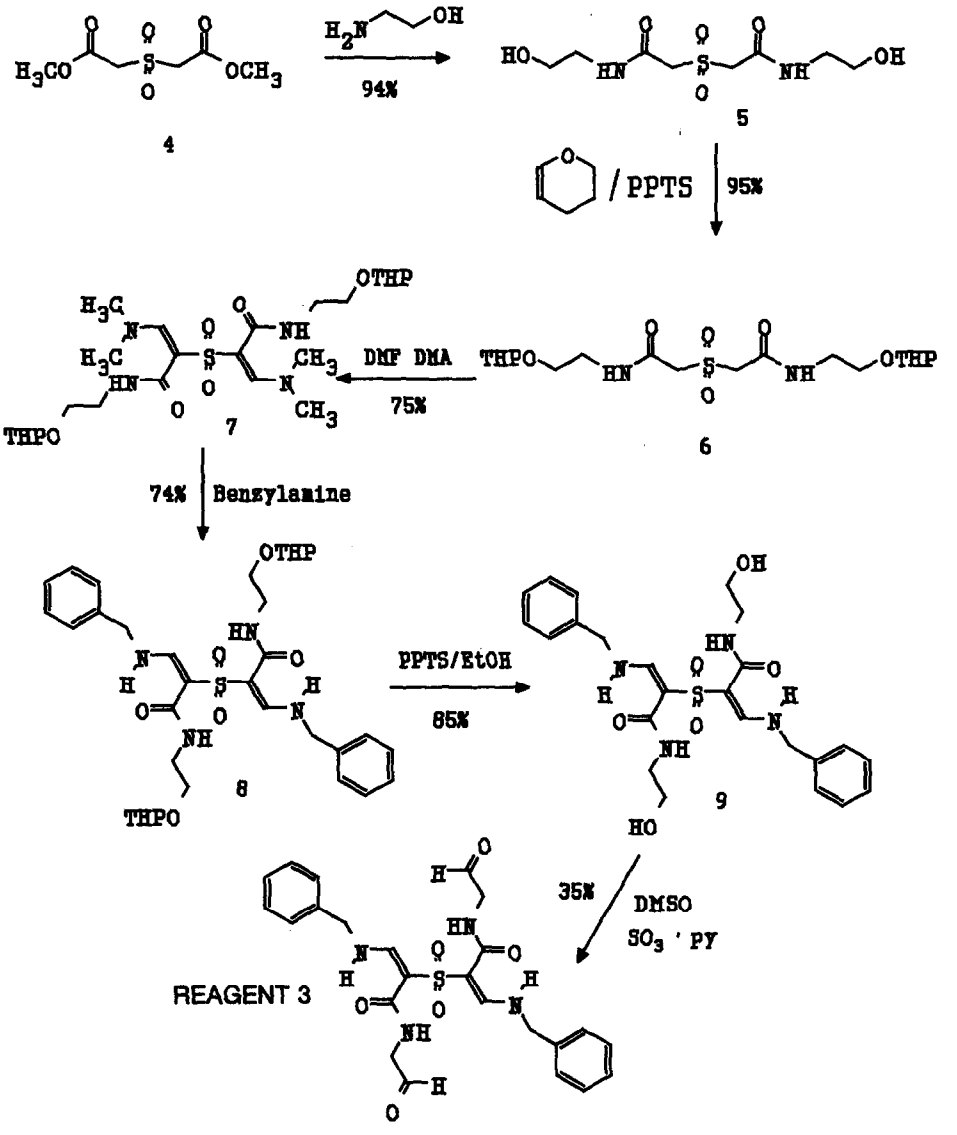
We now report the synthesis of a new bifunctional reagent **3** which is anticipated to eliminate the above two problems of distance and rigidity associated with reagents **1** and **2**. The reagent **3**, while still a bis-enamine, contains flexible bis-aldehydic groups as the new cross-linking sites, and has an estimated average tether length (A-A') ranging ~ 8.9 - 9.9 Å.[5] This length should be adequate to bridge the β_1-82 and β_2-82 lysine residues of human deoxyhemoglobin, which are separated by ~ 9.3 Å.[6] The bis-benzylamine arms of **3** are designed for later further manipulations, if necessary, of its hydrophobic as well as hydrophilic interactions (by appropriate substitutions on the phenyl ring) with the amino acids lining the periphery of the 2,3-diphosphoglycerate (DPG) pocket of hemoglobin tetramer.

The starting material for the synthesis of **3** (**Scheme**) is dimethyl 2,2'-sulfonyldiacetate (**4**)[7] which was reacted with ethanolamine to provide the corresponding bis(amide-alcohol) **5**.[8,9] The hydroxyl groups of **5** were protected with tetrahydropyranyl (THP) group by treatment with THP/pyridinium *p*-toluene-sulfonate (PPTS),[10] giving **6**.[8,9] The latter, upon treatment with dimethylformamide dimethyl acetal (DMF DMA), yielded the bis-enamine **7**.[8,9] The treatment of **7** with benzylamine afforded **8**,[8,9] which is consistent with our earlier observations[1c] that the disubstituted enamines undergo facile amine exchange reactions with primary amines to produce thermodynamically more stable enamines. Deprotection of the hydroxyl groups of **8** with alcoholic PPTS gave **9**.[8,9] Compound **9** was oxidized to **3**, employing Me_2SO/SO_3-pyridine complex.[11]

The reagent **3** is a white foam: ^{1}H NMR (Me_2SO-d_6): δ 9.56 (dt, 2H, J = 12.9, 6.3 Hz, ex. w/D_2O, enamine NHs), 9.40 (s, 2H, -CHOs), 8.06 (d, 2H, J = 13.7 Hz, s w/D_2O, =CHs), 7.63 (t, 2H, J = 4.5 Hz, ex. w/D_2O, amide NHs), 7.31 (s, 10H, phenyls), 4.49 (d, 4H, J = 6.1 Hz, s w/D_2O, benzylic CH_2s), 3.94 (d, 4H, J = 5.2 Hz, s w/D_2O, 2 CH_2-CO); IR (KBr): 3380 (amide NH), 1720 (CHO), 1628 (amide C=O), 1600 (C=C), 1524, 1449, 1363, 1313, 1273, 1230, 1127, 1082, 1013, 696, 632 cm^{-1}; MS (Cl w/*i*-C_4H_{10}): *m/z* 499 (MH^+, 55), 348 (26), 219 (23), 136 (100); UV λ_{max} (MeOH): 289.0 nm; *Anal.*[8] C, H, N, S.

Preliminary indication of potential utility of **3** as a cross-linking reagent was provided by its facile reactions with benzylamine, glycine methyl ester, and 2,4-dinitrophenylhydrazine, forming the corresponding bis-Schiff bases in nearly quantitative yields. Further exploration of its full potential as a biomacromolecular cross-linking agent is currently in progress.

SCHEME



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[5] The tether length of **3** was estimated *via* energy minimization, using ALCHEMY II™, a molecular modeling software by Tripos Associates, St. Louis, Missouri, **1988**.

[6] The estimated distance between the two lysine residues is based on the molecular modeling with CHARMm™ interfaced with QUANTA™ (Polygen Corp.), using X-ray co-ordinates of human hemoglobin obtained from the Brookhaven National Laboratory, Upton, Long Island, New York.

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[8] All compounds were analyzed for elements C, H, N, & S by Atlantic Microlab, Inc., Norcross, Georgia. The analyses were within ± 0.3% of calculated values.

[9] The physical and spectral data for the compounds are as follows: ***Compound 5***: colorless plate-like crystals, mp 165-166 °C; ^{1}H NMR (Me_2SO-d_6): δ 8.38 (br, 2H, ex. w/D_2O, amide NHs), 4.74 (t, 2H, J = 5.0 Hz, ex. w/D_2O, OHs), 4.25 (s, 4H, SO_2-CH_2s), 3.42 (ap q, 4H, J = 5.5 Hz, t w/D_2O, J = 5.5 Hz, CH_2-O), 3.17 (ap q, 4H, J = 5.5 Hz, m w/D_2O, N-CH_2s); IR (KBr): 3280 (amide NH), 3000, 2945, 2885, 1656 (C=O), 1550, 1432, 1410, 1328, 1292, 1212, 1140, 1055, 937, 910 cm^{-1}; MS (Cl w/i-C_4H_{10}) *m/z*: 269 (MH^+; 9.6), 251 (MH^+ - H_2O: 1.3), 182 (MH^+ - $C_3H_5NO_2$: 18), 88 ($HO(CH_2)_2HNCO^+$: 100). ***Compound 6***: colorless oil; ^{1}H NMR (Me_2SO-d_6): δ 8.44 (t, 2H, J = 4.4 Hz, ex. w/D_2O, NHs), 4.57 (t, 2H, J = 3.3 Hz, acetal CHs), 4.25 (s, 4H, SO_2-CH_2s, ex. w/D_2O), 3.75 (m, 2H), 3.63 (ap quintet, 2H, J = 5.2 Hz), 3.41 (m, 4H), 3.30 (m, 4H), 1.73 (m, 2H), 1.62 (dt, 2H, J = 9.2, 2.3 Hz), 1.47 (m, 8H); IR (KBr) 3450-3150, 3120-3040, 2942, 2866, 1665 (amide C=O), 1550, 1327, 1120, 1073, 1032 cm^{-1}; MS (Cl w/i-C_4H_{10}) *m/z* 437 (MH^+, 3.0), 353 (MH^+ - C_5H_8O, 21), 269 (MH^+ - $2C_5H_8O$, 35), 104 (100). ***Compound 7***: colorless crystals, mp 102-104 °C; ^{1}H NMR (Me_2SO-d_6): δ 7.59 (t, 2H, ex. w/D_2O, amide NHs), 7.06 (s, 2H, =CHs), 4.55 (br, 2H, acetal CHs), 3.5 (m, 12H, 2 N-CH_2-CH_2-O and 2 O-CH_2s), 2.92 (s, 12H, N(Me)$_2$s), 1.5 (br, 12H, 2 CH_2-CH_2-CH_2); IR (KBr): 3390 (amide NH), 2940, 2863, 1640 (C=O), 1596 (C=C), 1515, 1427, 1378, 1268, 1125, 1100, 1070, 1032, 653 cm^{-1}; MS (Cl w/i-C_4H_{10}): *m/z* 547 (MH^+, 44), 463 (MH^+ - C_5H_8O, 100), 379 (MH^+ - $2C_5H_8O$, 75), 85 ($C_5H_9O^+$, 23); UV λ_{max} (MeOH): 290.5 nm. ***Compound 8***: colorless thick oil; ^{1}H NMR (Me_2SO-d_6): δ 9.59 (dt, 2H, J = 13.8, 6 Hz, ex. w/D_2O, enamine NHs), 7.90 (d, 2H, J = 13.4 Hz, s w/D_2O, enamine =CHs), 7.32 (m, 12H, 2H ex. w/D_2O, phenyls and amide NHs), 4.53 (m, 6H, s w/D_2O, acetal CHs and benzylic CH_2s), 3.5 (m, 12H, 2 N-CH_2-CH_2-O and O-CH_2s of THPs), 2.0 (m, 12H, 2 CH_2-CH_2-CH_2); IR (KBr): 3375 (amide NH), 2940, 2865, 1630 (C=O), 1600 (C=C) cm^{-1}; MS (Cl w/i-C_4H_{10}): *m/z* 671 (MH^+, 12), 587 (MH^+ - C_5H_8O, 45), 503 (MH^+ - $2C_5H_8O$, 69), 85 ($C_5H_9O^+$, 100); UV λ_{max} (MeOH): 290.0 nm. ***Compound 9***: white solid, mp 165-166 °C; ^{1}H NMR (Me_2SO-d_6): δ 9.57 (dt, 2H, J = 13.6, 6 Hz, ex. w/D_2O, enamine NHs), 7.89 (d, 2H, J = 13.5 Hz, s w/D_2O, =CHs), 7.32 (m, 12H, 2H ex. w/D_2O, phenyls and amide NHs), 4.76 (t, 2H, J = 4.9 Hz, ex. w/D_2O, OHs), 4.50 (d, 4H, J = 6.1 Hz, s w/D_2O, benzylic CH_2s), 3.42 (m, 4H, O-CH_2s), 3.20 (m, 4H, amide N-CH_2s); IR (KBr): 3400 (amide NH), 1628 (C=O), 1521, 1350, 1273, 1233 cm^{-1}; MS (Cl w/i-C_4H_{10}): *m/z* 503 (MH^+, 100); UV λ_{max} (MeOH): 290.0 nm.

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