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A SHORT SERENDIPITOUS SYNTHESIS OF MINIMAL GLUCOCORTICOID RECEPTOR ZINC TEMPLATE

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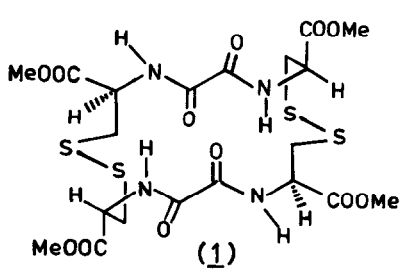
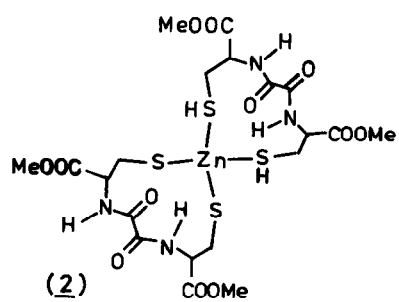
Abstract: The serendipitous formation of the novel, 20 membered, cyclo[bis-oxalyl cystine] - on treatment of cystine-di-OMe with oxalyl chloride - has provided an exceptionally short route to the first synthesis of the glucocorticoid receptor zinc template via PDT mediated thiol-disulfide exchange and metal complexation.

We have recently reported the first synthesis of a metal template which incorporated the essential features of the zinc finger template[1a] and a general strategy for the construction of zinc finger modules[1b]. Detailed studies have revealed that our template not only binds to induce ordered conformational changes with DNA and oligonucleotides, but also specifically so, when guanine residues are present[1c], thus simulating the action of zinc finger proteins[2]. These results provided impetus to prepare, *inter alia*, "left immobilized" zinc finger templates, having tethered amino ends in place of the short loop of zinc finger modules[2], to augment DNA interaction studies. With this objective, as the first step, cystine-di-OMe was reacted with oxalyl chloride.

Simultaneous slow addition of cystine-di-OMe and oxalyl chloride to stirred benzene admixed with triethylamine, under high dilution, afforded as the sole isolable product, compound (1), whose FAB mass spectra at once showed that it was not the expected 10 membered cyclo-oxalyl cystine, but the 20 membered cyclo[bis-oxalyl cystine] (1)[3,4]. This serendipitous discovery provided a direct route to the glucocorticoid receptor zinc template (2), thus making rational routes that were in progress to glucocorticoid receptor template motifs redundant.



(1)



(2)

Glucocorticoids, steroids and retinoids turn on the genes present in the cell nucleus by, complexation with receptor proteins (located at the cell surface), movement of the composite to the nucleus, recognition of specific DNA sequences and then promote transcription. A recent crystallographic study[5] has shown that the recognition helix in glucocorticoid receptor protein is preceded and followed by zinc template motifs, each arising from coordination with 4 invariable cysteines, the chirality of which around the central metal ion being different. The two zinc templates by contact with, DNA-sugar phosphate backbone and inter-subunits, bring about the precise alignment of the recognition helix; additionally the C-terminal template is actively involved in the dimerization of the receptor protein, a prerequisite for contiguous read out of 18 DNA base pairs.

As turned out to be the case with the zinc finger template motif[1c], we anticipated that DNA interaction studies with a basic glucocorticoid template motif, such as (2), will augment structural aspects pertaining to the interaction of these templates with DNA. We report the preparation of (2) in a single step, from the novel cyclo[bis-oxalyl cystine](1).

Compound (1), wherein 4 cysteines are interlocked by pairs of -S-S- bridges and -COCO- units, the orientations of which largely contribute to the overall conformation, represent an interesting system possessing many avenues for exploitation in the domain of protein design and recognition. Consequently, detailed studies were carried out, leading to the establishment of structure (1) for cyclo[bis-oxalyl cystine].

The appearance of, in the NH region of the 400MHz ^{1}H nmr, a single doublet centered at 8.13, 9.34 and 10.57 δ, in, respectively, $CDCl_3$, DMSO-d_6 and pyridine-d_5, clearly suggested that all the NH protons are solvent exposed, a conclusion that was further supported by solvent titration studies (gradient $CDCl_3$:DMSO-d_6 :: 0-40%; 24° C). The variable temperature (VT) 400MHz ^{1}H nmr of the region, monitered in the range of 247K - 343K afforded a $d\delta/dT$ value of -7.18 ppb/K, which clearly ruled out any intramolecular hydrogen bonding. The expected orthogonal disposition of the oxalamide units[6] is supported by the non-equivalence of the C_α as well as the ester carbonyl carbons in the proton decoupled 100MHz ^{13}C nmr.

The UV spectrum of (1) in EtOH showed λ_{max} at 250nm indicating an A type of transition of the disulfide chromophore close to a CSSC dihedral angle of $\pm$ 90° (P helical or M helical). The CD spectrum, also recorded in EtOH, exhibited at 265nm, a shallow negative ellipticity, thus showing a P helical mode of orientation[7]. Since each of the β-CH_2 protons appear as a clear set of double doublets, the helicity of both the disulfide bridges would have the same P helical conformation[8]. Thus, the disulfide bridges are PP helical with a dihedral angle of + 90°.

The spatial disposition of the oxalamide units discerned from nmr studies coupled with that of disulfide bridges from UV, CD measurements clearly establish structure (1) for cyclo[bis-oxalyl cystine].

This structural assignment is in agreement with an overwhelming body of evidence from crystallographic and solution studies, that the most stable conformation have a CSSC dihedral angle close to $+90^{o}$ (P helical)[9].

The (1) → (2) change was readily accomplished via propane-1,3-dithiol (PDT) mediated thiol-disulfide exchange[10], followed by complexation as reported previously[1a], but with added tetraethylammonium bromide[11].

The structural assignment for (2) is supported, *inter alia*, by FAB mass spectra, 400MHz nmr and comparison with similar templates available with us. A spectral comparison of (1) and (2) would show minimum structural perturbation in the dipeptide environment on metal complexation. This aspect has been noted previously on formation of similar templates by either procedure delineated here[1a] or upon reconstitution of template modules from metal-free precursors[12].

Cystine-di-O^{t}Bu has been similarly processed to analogs of (1) and (2), to provide carboxy de-protected substrates needed for DNA interaction studies.

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3. Triethylamine (1.63 ml, 11.74 mmol) was added to ice cooled and stirred suspension of cystine-di-OMe.2HCl (2.0g, 5.87 mmol) in methylenechloride (25ml), left stirred for 0.5h, admixed with ether (15ml), filtered, evaporated, dried, dissolved in dry benzene (75ml) and this solution was added simultaneously with a solution of oxalyl chloride (0.51ml, 5.87mmol) in dry benzene (75ml), very slowly, during 8h, to dry benzene (100ml) admixed with Et_3N (1.63ml, 11.74mmol). The reaction mixture was left stirred for 12h, filtered, washed with water (20ml), MeOH(10ml) and dried to afford 0.954g (53%) of crude (1), mp 180^{o}-185^{o}C, which was placed in Soxhlet thimble and extracted with EtOAc for 40h, followed by $CHCl_3$:MeOH::85:15 for 40h. The combined organic extracts on evaporation in vacuo gave 0.540g (30%) of pure (1) which on crystallisation from EtOAc-$CHCl_3$ afforded fine needles, mp 195^{o}-197^{o}C , $[\alpha]_D^{25}$ = -33.8^{o} (c = 0.142, $CHCl_3$) and gave analytical data in good agreement with expected values. ms: m/z 645.71 $(MH)^+$ (M^+ for $C_{20}H_{28}N_4O_{12}S_4$ calc. 644.70). The mass spectrum revealed the complete absence of cyclo[oxalyl cystine], M^+ calc. 322.35.

ir: ν_{max} (KBr) cm^{-1}: 3288, 1743, 1660, 1510.

^{1}H NMR (400 MHz) studies on (1) :

^{1}H NMR (δ):

i. $CDCl_3$, 24^{o}C: 3.22, 3.26 (4H, d,d, J = 16Hz,8Hz); 3.30, 3.34 (4H,d,d, J = 16Hz, 8Hz) (β-CH_2), 3.80(12H, s) (ester), 4.87(4H, t, J = 8.5Hz) (α-CH), 8.13 (4H, d, J = 8Hz), (NH).

ii. DMSO-d_6, 24^{o}C: 2.82, 2.86 (4H, d,d, J = 16Hz, 8Hz); 3.15, 3.19 (4H, d,d, J = 16Hz, 8Hz) (β-CH_2), 3.65 (12H,s)(ester), 4.59 (4H, d,d,J = 8Hz) (α-CH), 9.34 (4H, d, J = 8.48Hz)(NH).

iii.Pyridine-d_5, 24°C: 3.41 (8H,d,d, J = 8Hz,8Hz) (β-CH_2), 3.60(12H, s)(ester), 5.39 (4H,d,d,J = 8Hz,8Hz) (α-CH), 10.57 (4H, d, J = 8 Hz)(NH).

NH shift from solvent titration (gradient $CDCl_3$:DMSO-d_6): δ (% DMSO-d_6): 8.14 (0), 8.27 (2), 8.40 (4), 8.50 (6), 8.58 (8), 8.64 (10), 8.83 (18), 8.90 (20), 9.08 (40); shift Σ 0.94.

Temperature dependent NH shift in DMSO-d_6, δ (°C): 9.34 (24), 9.28 (30), 9.22 (40), 9.15 (50), 9.08 (60), 9.0 (70); $d\delta/dT$ ppb/K = -7.18.

^{13}C NMR (100 MHz), $CDCl_3$, 50°C: δ: 29.69 (β-CH_2), 52.85,52.97 (α-CH), 41.38 (OCH_3), 159.16,159.53 (COOMe), 159.64 (NHCO).

4. The reaction of cystine-di-OMe with succinyl chloride, precisely as described above, afforded only the novel 12 membered cyclosuccinyl cystine-di-OMe, mp 215°-220°C; FAB ms: m/z 351.41 $(MH)^+$.

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11. Preparation of zinc template (2): All operations were carried out under dry oxygen-free nitrogen blanket and dry, degassed solvents were used. To a stirred solution of (1) (0.087g, 0.135mmol) in MeOH (20ml) was added, 0.6M methanolic PDT (0.68ml, 0.406mmol). The reaction mixture was left stirred for 8h, filtered to remove undissolved materials, filtrate evaporated, thoroughly washed with benzene (3x5ml) to remove dithiolane polymer, dried to afford 0.055g (62.5%; 0.169mmol) of the thiol, mp 175°-177° C, which was taken up in MeOH (15ml), cooled to 0°, admixed with 0.72M methanolic solution of Et_3N (0.52ml, 0.372mmol), stirrred for 0.5h, admixed with 0.067M methanolic $ZnCl_2$ of 99.99%purity (1.20ml, 0.080mmol) followed by $(Et)_4N$ Br (0.027g, 0.165mmol), stirred for 0.75h, refrigerated overnight, centrifuged, the residue thoroughly washed with MeOH (2x10ml) to remove all extraneous compounds, and dried to afford 0.035g (overall yield : 35.6% from (1)) of (2) as a microcrystalline solid, mp 275°-280° C, which gave analytical data in good agreement with expected values.

ir: ν_{max} (KBr) cm^{-1} : 3376, 1740, 1683, 1575, 1506.

nmr: δ (DMSO-d_6) (400MHz): 1.01 (b, 2H)(-SH), 2.82 (m, 4H) 3.17 (m, 4H)(β-CH_2), 3.64 (s, 12H)(ester), 4.58 (m, 4H)(α-CH), 9.28 (d, J=8Hz, 4H)(-NH-).

ms: (FAB) m/z: 712 $(MH)^+$.

Natural glucocorticoid receptor template, as stated previously, can be represented as $[Zn(SR)_4]^{2-}$. It was felt that the transformation of (1) to template would offer product of the same profile and it was for this reason that Et_4NBr was used to provide the counter ion. However, conductivity measurements on (2) have clearly shown that it is a neutral molecule of the type, $[Zn(SR)_2(HSR)_2]$.

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