The Journal of Organic Chemistry

VOLUME 64, NUMBER 20 OCTOBER 1, 1999

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Articles

Use of the Dithiasuccinoyl (Dts) Amino Protecting Group for Solid-Phase Synthesis of Protected Peptide Nucleic Acid (PNA) Oligomers[1−3]

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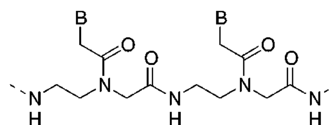
Received December 11, 1998 (Revised Manuscript Received July 9, 1999)

"Peptide nucleic acid" (PNA) oligomers replace the oligonucleotide backbone of DNA with an achiral and neutral poly[*N*-(2-aminoethyl)glycine] backbone, and the four natural nucleobases are attached through methylene carbonyl linkages to the glycine nitrogens. The present work describes the efficient conversion of N^{ω}-Boc/side-chain Z-protected PNA monomers to the corresponding derivatives protected by the thiolyzable N^{ω}-dithiasuccinoyl (Dts) function. After acidolytic removal of Boc, treatment with bis(ethoxythiocarbonyl) sulfide gave the N^{ω}-ethoxythiocarbonyl (Etc) derivatives, which were silylated at the α-carboxyl and converted to the heterocycle by reaction with (chlorocarbonyl)sulfenyl chloride. Net yields of homogeneous monomers were 71−78%. Conditions in the solid-phase mode for thiolytic removal of the Dts group, and for coupling of protected monomers, have been studied extensively and optimized. A protocol featuring (i) Dts removal with dithiothreitol (DTT) (0.5 M) in acetic acid (HOAc) (0.5 M)−CH_2Cl_2 (2 + 8 min); (ii) short neutralization with *N*,*N*-diisopropylethylamine (DIEA)−CH_2Cl_2 (1:19, 1 + 2 min); and (iii) coupling mediated by HBTU−DIEA (3:1) in *N*-methyl-2-pyrrolidinone (NMP) (3 h) was applied to the solid-phase synthesis of Dts-T_4-Gly-NH_2, Dts-G(Z)-G(Z)-T-A(Z)-Gly-NH_2, Dts-A(Z)-T-C(Z)-G(Z)-Gly-NH_2, and Dts-G(Z)-C(Z)-A(Z)-T-Gly-NH_2. The indicated protected PNA derivatives were released from the support, and their structures were verified by mass spectrometry.

Introduction

Peptide nucleic acids (PNAs) are DNA analogues with an achiral, uncharged pseudopeptide backbone composed of *N*-(2-aminoethyl)glycine monomer units; the nucleobases are attached to the glycine nitrogens via methylene carbonyl linkers (Scheme 1).[5] PNAs are chemically and biologically stable, and bind with high affinity and sequence specificity to both single-stranded DNA and RNA, as well as to double-stranded DNA. These properties make PNAs attractive leads for the development of gene therapeutics and biomolecular tools.[6] The prepara-

Scheme 1



B = nucleobases adenine, cytosine, guanine, and thymine

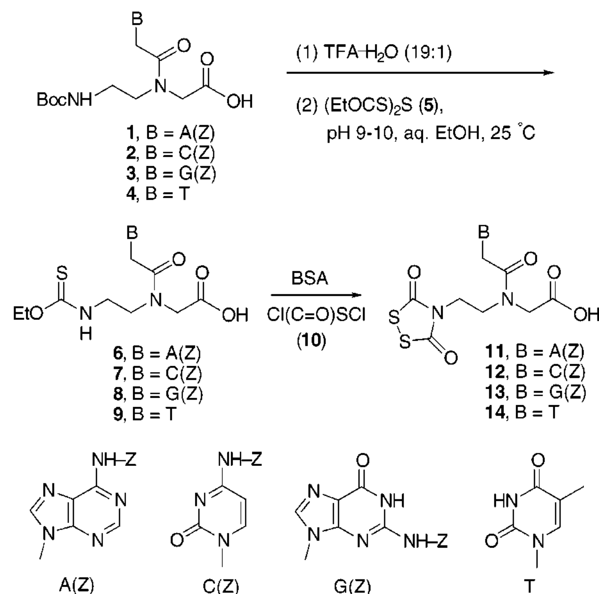
(1) Preliminary accounts of this work have been reported. (a) Working only with Dts-T-OH: Jensen, K. J.; Bardají, E.; Albericio, F.; Coull, J. M.; Barany, G. In *Peptides 1994*: *Proceedings of the Twenty-Third European Peptide Symposium*; Braga, Portugal, Sep 4−10, 1994; Maia, H. L. S., Ed.; ESCOM Science Publishers: Leiden, The Netherlands, 1995; pp 757−758. (b) Planas, M.; Bardají; Barany, G. In *Peptides 1998*: *Proceedings of the Twenty-Fifth European Peptide Symposium*; Budapest, Hungary, Aug 30−Sep 4, 1998; Bajusz, S., Hudecz, F., Eds.; Akadémia Kiadó: Budapest, Hungary, 1999; pp 234−235.

10.1021/jo9824394 CCC: $18.00 © 1999 American Chemical Society
Published on Web 09/08/1999

tion of PNA oligomers is based on standard solid-phase peptide synthesis protocols. Two different protection-schemes are currently used, Boc/Z[7] and Fmoc/Bhoc,[8] but the need for new and mild strategies remains.

The dithiasuccinoyl (Dts) function was developed to meet the requirements of an orthogonally removable N^{α}-amino protecting group,[9a] and has been studied thoroughly in our laboratories for the solid-phase synthesis of peptides.[9] The Dts group is stable to strong acids and photolysis but is rapidly and specifically removed under mild conditions by thiolysis.[9b] The present paper reports the application of the Dts group to the solid-phase synthesis of protected PNA oligomers. We describe

Scheme 2



procedures for the preparation of all four protected monomer building blocks, followed by studies to optimize conditions for deprotection/coupling cycles.

Results and Discussion

Preparation of N^{ω}-Dts-Protected Monomers. Introduction of the Dts moiety followed the general procedures described for the synthesis of Dts-protected amino acids and other Dts-protected amines (Scheme 2).[9] Starting from commercially available Boc-monomers **1**−**4**, the first step involved removal of the Boc group with TFA−H_2O (19:1), and this was followed immediately by treatment with bis(ethoxythiocarbonyl) sulfide[10] (**5**) in EtOH−H_2O (2:1), at pH 9−10, to provide the corresponding Etc analogues **6**−**9** in high yields and purities after straightforward workup. Freshly prepared **6**−**9**[11] were suspended in CH_3CN, treated with BSA (1.1−1.5 equiv) to effect trimethylsilylation at the α-carboxyl group and concomitant solubilization, and then treated in situ with (chlorocarbonyl)sulfenyl chloride (**10**) (1.1−1.2 equiv)[9c,11] to achieve conversion to the Dts-protected monomers **11**−**14**. These reactions proceed rapidly and in good overall yields (71−78%), with the caveat covered below.

The conversion of trimethylsilyl thiocarbamate derivatives of **6**−**9** to the corresponding Dts compounds **11**−**14**

(2) The following abbreviations are used: Ac_2O, acetic anhydride; βME, β-mercaptoethanol; Bhoc, benzhydryloxycarbonyl; Boc, *tert*-butyloxycarbonyl; BOP, benzotriazol-1-yl-*N*-oxytris(dimethylamino)-phosphonium hexafluorophosphate; BSA, *N,O*-bis(trimethylsilyl-acetamide); DECA, *N,N*-diethylcyclohexylamine; DIEA, *N,N*-diisopropylethylamine; DIPCDI, *N,N'*-diisopropylcarbodiimide; DMAP, *N,N*-dimethyl-4-aminopyridine; DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide; Dts, dithiasuccinoyl; DTT, dithiothreitol; Etc, ethoxythiocarbonyl; Et_2O, diethyl ether; EtOAc, ethyl acetate; EtOH, ethanol; FABMS, fast-atom bombardment mass spectrometry; Fmoc, 9-fluorenylmethoxycarbonyl; HAL, 5-(4-hydroxymethyl-3,5-dimethoxyphenoxy) valeric acid handle; HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HBTU, *N*-[1*H*-(benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HOAc, acetic acid; HOAt, 7-aza-1-hydroxybenzotriazole (3-hydroxy-3*H*-1,2,3-triazolo-[4,5-*b*]pyridine); HOBt, 1-hydroxybenzotriazole; HPLC, high-performance liquid chromatography; IRAA, "internal reference" amino acid; MAc, *N*-methylmercaptoacetamide; MSNT, 1-(mesitylene-2-sulfonyl)-3-nitro-1*H*-1,2,4-triazole; NMM, *N*-methylmorpholine; NMP, *N*-methyl-2-pyrrolidinone; PAL, 5-[[(4-amino)-methyl]-3,5-dimethoxyphenoxy]valeric acid handle (Peptide Amide Linker); PEG-PS, poly(ethylene glycol)-polystyrene (graft resin support); TBTU, *O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid; TFFH, 1,1,3,3-tetramethyl-2-fluoroformamidinium hexafluorophosphate; Z, benzyloxycarbonyl. All solvent ratios are volume/volume unless stated otherwise.

(3) In denoting PNA structures, both monomer building blocks and oligomers, standard oligopeptide nomenclature is used. For example, in the oligomer Dts-G(Z)-C(Z)-A(Z)-T-Gly-NH_2, G, C, A, and T are the G-acetyl, C-acetyl, A-acetyl,and T-acetyl *N*-(2-aminoethyl)glycyl units, respectively; Gly-NH_2 is the *C*-terminal glycinamide; Dts denotes the *N*-terminal amino protecting group; and, Z is the *N*-amino protecting group of the nucleobases. We also point out that while technically PNA molecules are neutral rather than acidic, workers in the field have retained the term "acid" in the name to emphasize the analogy to DNA and RNA.

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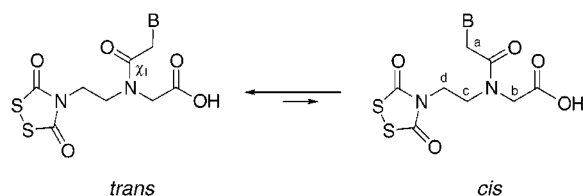
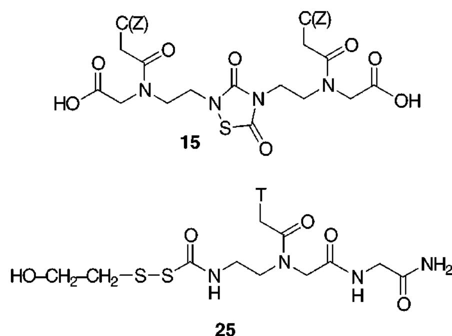
(11) Compounds **6**−**9** should be prepared freshly because they are prone to rearrange to the thermodynamically more stable *S*-alkyl thiocarbamate isomers under a variety of conditions, including prolonged standing. Although 1 equiv of (chlorocarbonyl)sulfenyl chloride (**10**) is sufficient for all of the Etc derivative to be converted to products, the protocols reported here with (chlorocarbonyl)sulfenyl chloride (**10**) always in excess were designed to minimize the isomerization problem. See ref 9c.

Scheme 3[a]



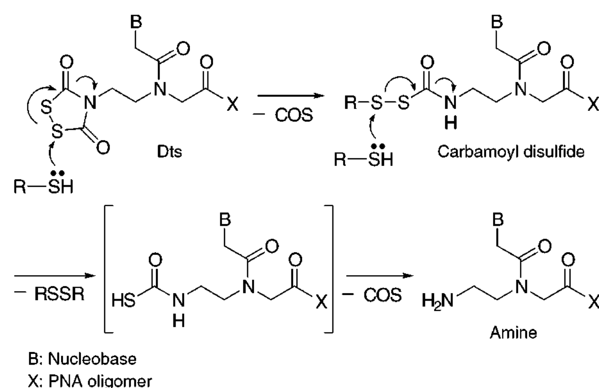
[a] Rotamers of Dts-PNA monomers. The trans configuration is preferred. When the protecting group on N^{ω} is Boc or Etc (structures not shown), the preference is the opposite (see text).

was usually carried out under ambient conditions, but this mode gave problems when the target was Dts-C(Z)-OH (**12**). Despite varying the ratio of **7**:**10**, the product mixture always included a substantial amount (as much as 27% of the crude reaction mixture) of a byproduct in addition to the expected **12**. LC/MS analysis suggested that the byproduct was the 1,2,4-thiadiazolidine-3,5-dione heterocycle **15**. Such heterocycles were identified previously in studies of Dts formation in simpler systems.[9c] Here, formation of **15** was suppressed entirely by carrying out the reaction of **10** with the trimethylsilyl ester of **7** at 0 °C.



The ^{1}H and ^{13}C NMR spectra of Etc and Dts monomers were consistent with the presence of two rotamers. Similar observations were reported previously[12] for other PNA monomers, as well as oligomers, and have been ascribed to slow chemical exchange due to a cis–trans equilibrium about the tertiary amide bond (torsion angle χ_1) (Scheme 3). To assign conformations of the amide group, we first confirmed the literature conclusion from 1D and 2D ^{1}H NMR experiments that in the Boc-monomers **1**–**4** the side-chain carbonyl group of the major rotamer points toward the C terminus[12a,13] (cis conformation, Scheme 3). Thus, in a 2D NOESY spectrum, cross-peaks between protons a and c–d were observed for the major rotamer, while the minor conformer revealed cross-peaks between protons a and b. Similar results were observed with the Etc intermediates. In contrast, the Dts-monomers showed peak intensities opposite to Boc and Etc, suggesting that Dts protection confers a preference for trans. This was confirmed by 2D NOESY experiments, where cross-peaks between protons

Scheme 4[a]



16, R–SH = $CH_3OC_6H_4CH_2$–SH
17, R–SH = 1,2–$C_6H_4(CH_2$–$SH)_2$
18, R–SH = $C_6H_5CH_2$–SH
19, R–SH = $HOCH_2CH_2$–SH (βME)
20, R–SH = $(CHOHCH_2$–$SH)_2$ (DTT)
21, R–SH = $CH_3NH(CO)CH_2$–SH(MAc)
22, R–SH = $CH_3C_6H_4$–SH
23, R–SH = C_6H_5–SH
24, R–SH = ClC_6H_4–SH

[a] Thiols and dithiols are listed in order of pK_a (see Tables 1 and 2).

a and b observed for the major rotamer indicates that methylene protons of the acyl side chain are close to the glycine α-protons. The cis:trans ratio varies depending on the nucleobase side-chain; values were 1:2 for Dts-A(Z)-OH (**11**), 3:4 for Dts-C(Z)-OH (**12**), 1:2 for Dts-G(Z)-OH (**13**), and 1:1.5 for Dts-T-OH (**14**).

Solid-Phase Deprotection Studies. The Dts protecting group is removed by thiols[9b,14] through the intermediacy of an open-chain carbamoyl disulfide, which reacts further with a second equivalent of the thiol to give the free amino function (Scheme 4). The reaction is driven to completion by loss of 2 equiv of gaseous carbonyl sulfide, and is usually catalyzed by tertiary amines. Optimized protocols for quantitative thiolytic removal of the Dts group[9d,e] during solid-phase peptide synthesis include (i) βME (0.5 M)–DIEA (0.5 M) in CH_2Cl_2, 2 × 2 min; (ii) MAc (0.5 M)–NMM (0.5 M) in CH_2Cl_2, 2 × 2 min; or (iii) MAc (0.5 M)–HOBt (0.1 M) in DMF, 2 × 2 min. When conditions (i) were applied to the solid-phase synthesis of Dts-T_5-Gly-NH_2, the main product was the expected one, but several byproducts were also noted.[1a,15] For the present studies, we considered a variety of thiols over a 4-order-of-magnitude pK_a range,[16] including aliphatic, aromatic, and bifunctional thiols, and focused on the development of deprotection conditions that could be applied in the absence of base and/or the presence of acid (Scheme 4, Tables 1 and 2).

Dts-T-Gly-PAL-PEG-PS served as a model system to investigate deprotection rates and pathways; the glycine residue was incorporated to facilitate solubility and quantification. The Dts-resin was treated with the ap-

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(13) Reference 12c claims that in the major rotamer of the Boc monomers the tertiary amide bond has a trans conformation, but these results do not agree with ref 12a or with our own experiments.

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(15) In the preliminary work (ref 1a), formation of thiourethanes due to βME were detected by HPLC and characterized by FABMS and LC/MS. In the present work, with improved conditions, the thiourethanes were not observed.

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Table 1. Representative Solid-Phase Deprotections of Dts-T-Gly-PAL-PEG-PS[a]

entry	deprotection conditions[b]			product distribution (% HPLC)[c]				
	thiol[d]	additive/solvent	time (min)	H-T-Gly	**26**	Dts-T-Gly	thiourethane	**25**
1	**16**[e]	NMP	1	44	36		3	
2	**16**	HOAc (0.1 M)−NMP	1	61	23		3	
3	**16**	HOBt (0.1 M)−NMP	1	36	37		3	
4	**16**	CH_2Cl_2	1 + 2 + 2	38	5	20		
5	**16**	HOAc (0.1 M)−CH_2Cl_2	1 + 2 + 2			79		
6	**17**	HOAc (0.1 M)−NMP	1 + 2 + 2	55	27			
7	**18**[f]	HOAc (0.1 M)−NMP	1 + 2 + 2	52	31		7	
8	**19**[g]	HOAc (0.1 M)−CH_2Cl_2	1 + 2 + 2	69	18			
9	**19**	HOAc (0.5 M)−CH_2Cl_2	2 + 8	10	6	9		67
10	**21**[h]	HOAc (0.5 M)−CH_2Cl_2	2 + 8	89	1			
11	**22**[i]	HOAc (0.1 M)−CH_2Cl_2	1 + 2 + 2	38	27	4	5	
12	**23**[j]	HOAc (0.1 M)−CH_2Cl_2	1 + 2 + 2	42	25	3	3	
13	**24**[k]	HOAc (0.1 M)−CH_2Cl_2	1 + 2 + 2	38	23	7	4	

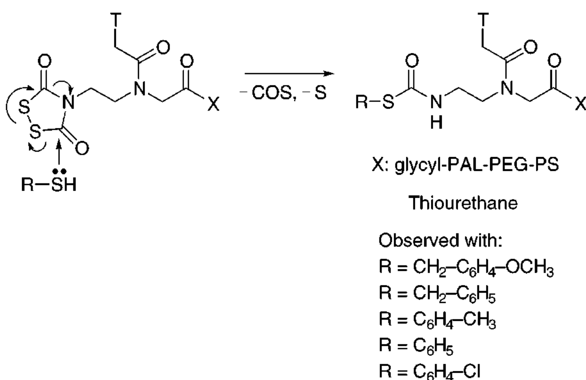
[a] Excerpted from extensive tables (1−10) in the Supporting Information. [b] See the Experimental Section for general procedures. [c] Relative amounts of species reported are based on uncorrected total HPLC values, and do not necessarily add to 100%. [d] [Thiol] = 0.5 M. [e] pK_a = 9.86 (ref 16a). [f] pK_a = 9.55 (ref 16a). [g] pK_a = 9.45 (ref 16a). [h] pK_a = 8.0 (ref 16b). [i] pK_a = 6.8 (ref 16c). [j] pK_a = 6.6 (ref 16c). [k] pK_a = 6.1 (ref 16c).

Table 2. Solid-Phase Deprotections of Dts-T-Gly-PAL-PEG-PS with DTT (20)[a]

entry	deprotection conditions[b]				product distribution (% HPLC)[c]		
	[DTT][d] (M)	loading (mmol/g)	additive/solvent	time (min)	H-T-Gly	**26**	Dts-T-Gly
1	0.5	0.18	HOAc (0.1 M)−NMP	1 + 2 + 2	75	15	
2	0.5	0.18	HOBt (0.1 M)−NMP	1 + 2 + 2	70	15	
3	0.5	0.18	NMP	1 + 2 + 2	68	16	
4	0.5	0.18	CH_2Cl_2	1 + 2 + 2	73	11	
5	1	0.18	HOBt (0.1 M)−CH_2Cl_2	1 + 2 + 2	73	16	
6	0.5	0.18	HOAc (0.1 M)−CH_2Cl_2	1 + 2 + 2	76	12	
7	0.5	0.09	HOAc (0.1 M)−CH_2Cl_2	1 + 2 + 2	82	1	
8	0.5	0.18	HOAc (0.5 M)−CH_2Cl_2	2 + 8	90	1	
9	0.5	0.18	HOAc (1 M)−CH_2Cl_2	2 + 8	91		

[a] Excerpted from extensive tables (1−10) in the Supporting Information. [b] See the Experimental Section for general procedures. [c] Relative amounts of species reported are based on uncorrected total HPLC values and do not necessarily add to 100%. [d] pK_a = 8.6 (ref 16b).

Scheme 5

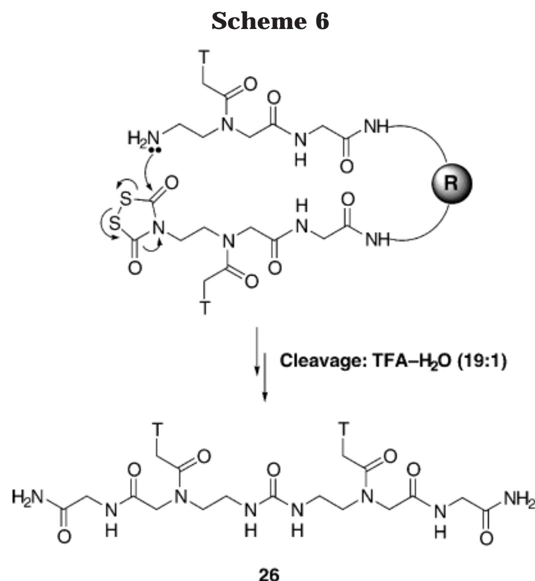


propriate thiol for a particular time period, and then cleaved with TFA−H_2O (19:1, 2 h); the crude product mixture was then dissolved in H_2O and analyzed by HPLC and mass spectrometry. The desired major product from deprotection, H-T-Gly-NH_2, was characterized by mass spectrometry and co-injected with a sample prepared by an Fmoc strategy. With certain thiols under some solvent conditions, relatively low levels of thiourethane byproducts were observed (Scheme 5, Table 1, entries 1−3, 7, and 11−13). As a control, the resin when cleaved directly (without deprotection) provided Dts-T-Gly-NH_2, which was characterized further by mass spectrometry and ^{1}H NMR. Finally, in some experiments using βME (Table 1, entry 9) for deprotection, the carbamoyl disulfide intermediate **25** was observed.

With all thiols tested, the Dts function was rapidly transformed when NMP, HOAc (0.1 M)−NMP, or HOBt (0.1 M)−NMP was used as solvent (e.g., Table 1, entries 1−3; no starting material was observed after 1 min treatment). However, when CH_2Cl_2 or HOAc (0.1 M)−CH_2Cl_2 was used as solvent, reactions were slower as indicated by observation of unreacted Dts-T-Gly-NH_2 in some cases (e.g., Table 1, compare entries 1 and 4). These observations agree with previous results that Dts thiolysis is promoted in polar aprotic media with high dielectric constants.[9b,16b] A common byproduct during these reactions was invariably noted, irrespective of the thiol used, and accounted for as much as 42% of the product mixture. The material was isolated and analyzed by FABMS, from which the urea structure **26** was proposed. Formation of **26** is plausibly explained by an intersite nucleophilic attack of the amino terminus from an already-deprotected H-T-Gly resin-bound unit onto the carbonyl of the Dts group of another still-protected resin-bound unit, with ultimate loss of carbonyl sulfide and elemental sulfur (Scheme 6). The susceptibility of the Dts heterocycle to aminolysis is precedented,[9b,e] although this specific side reaction has not been described earlier.

Based on the understanding of the source of byproduct **26**, two strategies were devised to suppress its formation. First, by working with resins of lower loading, the effective concentration of reactive sites was expected to be reduced. Indeed, the use of DTT (0.5 M) in HOAc (0.1 M)−CH_2Cl_2 to deprotect Dts-T-Gly-PAL-PEG-PS gave 12% of **26** at 0.18 mmol/g loading, but only 1% of **26** at 0.09 mmol/g loading (Table 2, compare entries 6 and 7). Second, by increasing the acidity of the solvent milieu for deprotection, it was expected that the liberated amino groups after Dts removal would be protonated and less

Scheme 6



likely to serve as nucleophiles. Again, this prediction was borne out experimentally, particularly with MAc or DTT as thiols (Table 1, entry 10; Table 2, compare entry 6 with 8 and 9). The greater acidity made it necessary to apply longer treatments to achieve complete Dts removal. Suitable conditions for quantitative removal of the Dts-amino protecting group at 25 °C, estimated on the basis of the considerations outlined in the previous paragraphs, include (i) DTT or MAc (0.5 M) in HOAc (0.5 M)$-CH_2Cl_2$ for 2 + 8 min; (ii) DTT (0.5 M) in HOAc (1 M)$-CH_2Cl_2$ for 2 + 8 min. These conditions were studied further, as described in the next section.

Evaluation of Conditions for Solid-Phase Synthesis of Dts-T_4-Gly-NH_2. Initial studies focused on the model Dts-T_4-Gly-NH_2 (Table 3). Optimized conditions for Dts removal were applied, and a *short* neutralization step with DIEA$-CH_2Cl_2$ (1:19, 1 + 2 min) was interpolated to follow the deprotection. Couplings of the Dts-T-OH monomer were mediated by a variety of reagent/additive protocols, always with NMP as solvent. After chain assembly was completed, the Dts-protected tetramers were cleaved from the resin with TFA$-H_2O$ (19:1), and the crude product was analyzed by HPLC and mass spectrometry.

For syntheses of PNA by Boc chemistry, HATU and HBTU with in situ neutralization were reported to give the best results.[7e,f] However, standard coupling protocols with aminium salts require a tertiary amine for optimal efficiency,[17] leading to a concern that the applicability of such methods to Dts chemistry might be compromised by the known lability of the Dts group to amines. Activation at each step of Dts-T-OH (3 equiv) with HBTU/DIEA (3:3) provided crude Dts-T_4-Gly-NH_2 with substantial heterogeneity (Table 3, entries 1 and 3). Results did not improve upon changing to a weaker base, such as collidine (Table 3, entry 2). Also, a combination of the BOP reagent[18]/DIEA (3:3) was tested, giving similar values (Table 3, entry 12). However, substantially better results were achieved by reducing the amount of tertiary amine during the coupling, i.e., HBTU/DIEA (3:1); this led to Dts-T_4-Gly-NH_2 of 80% purity (Table 3, entry 5) (Figure 1a). When NMM, DMAP, or DECA was used with similar ratios, results were inferior (Table 3, entries 7−9). Omission of base entirely led to no product (Table 3, entries 10 and 15). Generation of in situ acyl fluorides with TFFH[19]/DIEA (3:1) was not successful (Table 3, entry 17), nor was the classical DIPCDI/HOBt method (Table 3, entry 11). Finally, results with HBTU were slightly better than those with TBTU, HATU, or BOP (Table 3, compare entries 13, 14, and 16).

In summary, a repetitive deprotection/coupling cycle for incorporation of a Dts-PNA monomer at 25 °C involves: (i) thiolytic deprotection with DTT (0.5 M) in HOAc (0.5 M)$-CH_2Cl_2$, 2 + 8 min; (ii) neutralization with DIEA$-CH_2Cl_2$ (1:19, 1 + 2 min), and (iii) coupling of Dts-PNA-monomer (3 equiv) using HBTU/DIEA (3:1) in NMP, 3 h.

Solid-Phase Synthesis of Dts-Protected PNA Tetramers. The methodology summarized above for Dts-T_4-Gly-NH_2, was extended to the synthesis of Dts-protected tetramers containing the four nucleobases. Thus, Dts-G(Z)-G(Z)-T-A(Z)-Gly-NH_2 (54% purity, Figure 1b), Dts-A(Z)-T-C(Z)-G(Z)-Gly-NH_2 (54% purity, Figure 1c), and Dts-G(Z)-C(Z)-A(Z)-T-Gly-NH_2 (62% purity, Figure 1d) were prepared and characterized by LC/MS.

Conclusions

We have described an efficient procedure for preparation of Dts-protected PNA monomers and defined protocols for their application to the synthesis of Dts-protected oligomers under *mild*, orthogonal conditions. Since Dts removal can be achieved under acidic conditions followed by a short neutralization step, previously described PNA synthesis side reactions[6b,f,8] such as *N*-acyl migration or *N*-terminal deletion products, might be minimized in this system. Ongoing work is directed at the use of protected tetramers as building blocks[20] for segment condensation synthesis of longer PNA.

Experimental Section

Materials, solvents, instrumentation, and general methods were essentially as described in previous publications from our laboratories.[9,10,14] HATU, PEG-PS resins[23] (0.15−0.18 mmol/g and containing Nle as the IRAA), protected PNA monomers, and TFFH were obtained from the BioSearch division of PerSeptive Biosystems (Framingham, MA). DIEA, HOBt, NMP, piperidine, and TFA were from Fisher (Pittsburgh, PA).

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(18) Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, 1219−1222.

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(20) Ideally, these building blocks should have a free carboxyl group. Toward this end, we synthesized Boc-T_4-OH at a stage in the research program where other aspects had not been optimized. Dts-T-OH (2 equiv) was anchored onto HAL-PEG-PS (0.20 g, 0.23 mmol/g) (ref 21) by a protocol including MSNT (2 equiv) (ref 22) activation in the presence of *N*-methylimidazole (5 equiv) in CH_2Cl_2−THF (1:2, 3 mL). Deprotection/couplings introduced two further Dts-T-OH, and the terminal T was introduced with N^{ω}-Boc protection. The protected PNA tetramer was released from the HAL anchor with TFA−CH_2Cl_2 (1:999, 2 mL) for 2 h at 25 °C and characterized by HPLC (>60% purity) and ESMS (calcd for $C_{49}H_{66}N_{16}O_{19}$ 1182.5, found *m/z* 1205.4 [M + Na]$^+$).

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(22) Nielsen, J.; Lyngsø, L. O. *Tetrahedron Lett.* **1996**, 8439−8442.

Table 3. Evaluation of Conditions for Solid-Phase Synthesis of Dts-T_4-Gly-NH_2[a]

	coupling conditions[b]		deprotection conditions			
entry	reagent	base or additive	thiol	solvent	time (min)	Dts-T_4-Gly-NH_2[c]
1	HBTU (3 equiv)	DIEA (3 equiv)	DTT (0.5 M)	HOAc (1 M)−CH_2Cl_2	2 + 15	46
2	HBTU (3 equiv)	collidine (3 equiv)	DTT (0.5 M)	HOAc (1 M)−CH_2Cl_2	2 + 15	36
3	HBTU (3 equiv)	DIEA (3 equiv)	DTT (0.5 M)	HOAc (0.5 M)−CH_2Cl_2	2 + 8	57
4	HBTU (3 equiv)	DIEA (1 equiv)	DTT (0.5 M)	HOAc (1 M)−CH_2Cl_2	2 + 8	68
5	HBTU (3 equiv)	DIEA (1 equiv)	DTT (0.5 M)	HOAc (0.5 M)−CH_2Cl_2	2 + 8	80
6	HBTU (3 equiv)	DIEA (1 equiv)	MAc (0.5 M)	HOAc (0.5 M)−CH_2Cl_2	2 + 8	70
7	HBTU (3 equiv)	NMM (1 equiv)	DTT (0.5 M)	HOAc (0.5 M)−CH_2Cl_2	2 + 8	36
8	HBTU (3 equiv)	DMAP (1 equiv)				NP[d]
9	HBTU (3 equiv)	DECA (1 equiv)	DTT (0.5 M)	HOAc (0.5 M)−CH_2Cl_2	2 + 8	55
10	HBTU (3 equiv)					NP[d]
11	DIPCDI (3 equiv)	HOBt (3 equiv)				NP[d]
12	BOP (3 equiv)	DIEA (3 equiv)	DTT (0.5 M)	HOAc (1 M)−CH_2Cl_2	2 + 15	45
13	BOP (3 equiv)	DIEA (1 equiv)	DTT (0.5 M)	HOAc (0.5 M)−CH_2Cl_2	2 + 8	67
14	TBTU (3 equiv)	DIEA (1 equiv)	DTT (0.5 M)	HOAc (0.5 M)−CH_2Cl_2	2 + 8	69
15	TBTU (3 equiv)					NP[d]
16	HATU (3 equiv)	DIEA (1 equiv)	DTT (0.5 M)	HOAc (0.5 M)−CH_2Cl_2	2 + 8	60
17	TFFH (3 equiv)	DIEA (1 equiv)	DTT (0.5 M)	HOAc (0.5 M)−CH_2Cl_2	2 + 8	15

[a] See the Experimental Section for general procedures. [b] Dts-T-OH (3 equiv); solvent, NMP; time, 3 h. Upon completion of coupling, ninhydrin test was negative. [c] Percent determined by HPLC. [d] No product was formed; syntheses were aborted after the first coupling step, since positive ninhydrin tests indicated that coupling was incomplete.

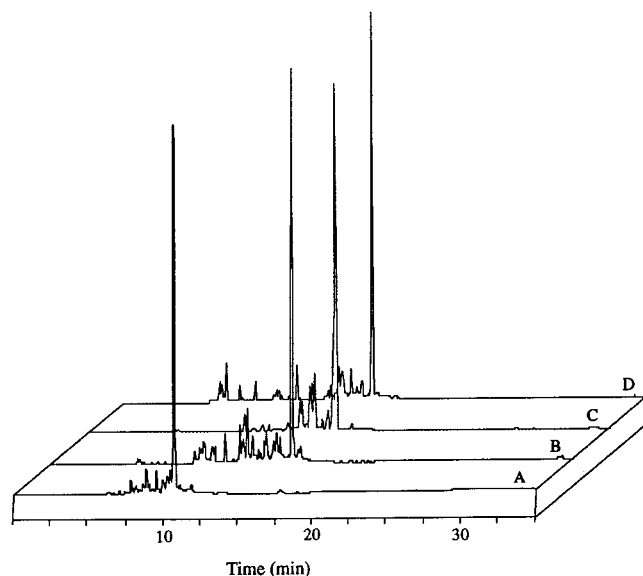

Figure 1. Analytical HPLC of crude product from synthesis of (A) Dts-T_4-Gly-NH_2, (B) Dts-G(Z)-G(Z)-T-A(Z)-Gly-NH_2, (C) Dts-A(Z)-T-C(Z)-G(Z)-Gly-NH_2, and (D) Dts-G(Z)-C(Z)-A(Z)-T-Gly-NH_2. Chromatography followed conditions listed in the Experimental Section. Ordinate is absorbance at 265 nm.

o-Benzenedimethanethiol, benzylmercaptan, βME, BSA, DECA, DIPCDI, DMAP, DTT, *p*-chlorothiophenol, collidine, *p*-methoxybenzylmercaptan, *p*-methylthiophenol, pyridine, and thiophenol were from Aldrich (Milwaukee, WI), and MAc was obtained from Fluka (Ronkonkoma, NY). BOP, HBTU, and TBTU were from Advanced ChemTech (Louisville, KY). Bis(ethoxythiocarbonyl) sulfide (**5**) was prepared as described previously,[10] or the product purchased from Fairfield Chemical (Blythewood, SC) was recrystallized from ethanol−water (3:1). (Chlorocarbonyl)sulfenyl chloride (**10**) was prepared as described previously.[24]

All transformations (solution and solid-phase) and washes were done at 25 °C unless noted otherwise. PNA-resin samples were hydrolyzed in 12 N aqueous HCl−propionic acid (1:1) at 160 °C for 2 h, and analyses for Gly and NLeu were carried out on an amino acid analyzer. Melting points are uncorrected. Silica gel chromatography was performed with silica gel 60 (230−400 mesh) from EM Science (Gibbstown, NJ). Thin-layer chromatography was performed on either Polygram SIL G/UV_{254} plates (250 mm, 40 × 80 mm, Macherey-Nagel) or Kieselgel 60 F_{254} (0.2 mm, 40 × 80 mm, EM Science); spots were visualized by UV. Elemental analyses were conducted by M−H−W Laboratories, Phoenix, AZ.

Analytical HPLC was performed at 1.2 mL/min using a Vydac C_{18} reversed-phase column (0.46 × 25 cm; 5 μm particle size) with UV detection at 220 and 265 nm. Linear gradients of 0.1% aqueous TFA and 0.1% TFA in CH_3CN were run from 1:0 to 1:1 over 15 min and then 1:1 to 1:3 over 5 min. Low-resolution fast-atom bombardment mass spectroscopy (FABMS) was carried out in glycerol−H_2O or 3-nitrobenzyl alcohol (MNBA) matrices on a high-resolution double-focusing mass spectrometer operated at a resolution of 2000. Liquid chromatography/mass spectrometry (LC/MS) was performed using a Zorbax SB-C_{18} narrowbore reversed-phase column (3.0 mm × 25 cm; 5 μm particle size) and detection at 220 nm. This system was connected to a PE-Sciex API *III* triple-quadrupole mass spectrometer equipped with an ionspray interface.

^{1}H NMR spectra were recorded at 300 MHz or at 500 MHz. Assignments of spectra were based on 2D homonuclear chemical-shift correlation spectroscopy. 2D NOESY spectra were acquired at 298 K with 300 ms mixing time. ^{13}C NMR spectra were recorded at 75 or 125 MHz, and assignments were based on heteronuclear multiple quantum coherence (HMQC) spectroscopy.

***N*-[2-(*N*-Ethoxythiocarbonyl)aminoethyl]-*N*-[[6-*N*-(benzyloxycarbonyl)adenin-9-yl]acetyl]glycine (6).** A solution of Boc-A(Z)-OH (**1**) (409 mg, 0.78 mmol) in TFA−H_2O (19:1, 16 mL) was stirred for 60 min. Concentration in vacuo (1 mm) gave an oil that was redissolved in EtOH−H_2O (2:1) (15 mL). Next, bis(ethoxythiocarbonyl) sulfide (**5**) (246 mg, 1.17 mmol, 1.5 equiv) was added and the resulting suspension brought to a pH of 9−10 by addition of 2.5 M aqueous NaOH (2.0 mL). Additional 2.5 M aqueous NaOH (0.6 mL) was added dropwise over a period of 4 h in order to maintain the pH; at this point, all of **5** was dissolved and the pH remained constant overnight. The solution was diluted with H_2O (18 mL), acidified to pH 2 with 12 N HCl, and extracted with EtOAc (5 × 20 mL). The organic layers were combined, dried ($MgSO_4$), and evaporated in vacuo to provide a colorless oil. Trituration

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(24) Barany, G.; Schroll, A. L.; Mott, A. W.; Halsrud, D. A. *J. Org. Chem.* **1983**, *48*, 4750−4761.

of the oil with Et_2O yielded **6** as a white solid that was dried over P_2O_5 (385 mg, 95%): mp 118–120 °C; ^1H NMR (DMSO-d_6) δ (two rotamers) 9.26 (m, 0.6H), 9.02 (m, 0.4H), 8.58 (s, 1H), 8.33 (s, 0.4H), 8.32 (s, 0.6H), 7.45 (d, J = 7.0 Hz, 2H), 7.38 (d, J = 7.0 Hz, 2H), 7.33 (t, J = 7.0 Hz, 1H), 5.36 (s, 1.2H), 5.21 (s, 2H), 5.16 (s, 0.8H), 4.41 (q, J = 7.0 Hz, 1.2H), 4.36 (s, 0.8H), 4.35 (q, J = 7.0 Hz, 0.8H), 4.01 (s, 1.2H), 3.70–3.34 (m, 4H), 1.26 (t, J = 7.0 Hz, 1.8H), 1.20 (t, J = 7.0 Hz, 1.2H); ^{13}C NMR (DMSO-d_6) δ[25] (190.3, 189.9), (170.8, 170.3), (167.2, 166.6), 152.4, 152.2, 151.5, 149.3, 145.2, 136.4, 128.4, 128.0, 127.9, 122.8, 66.3 (65.6, 65.3), (49.2, 47.6), (45.7, 45.4), (44.1, 43.9), (42.7, 41.9), (14.2, 14.1); FABMS m/z 516.2 $[M + H]^+$, 514.1 $[M - H]^-$. Anal. Calcd for $C_{22}H_{25}N_7O_6S{\cdot}{}^3/_2H_2O$: C, 48.70; H, 5.16; N, 18.07; S, 5.91. Found: C, 48.57; H, 4.88; N, 17.79; S, 5.20.

***N*-[2-(*N*-Ethoxythiocarbonyl)aminoethyl]-*N*-[[4-*N*-(benzyloxycarbonyl)cytosin-1-yl]acetyl]glycine (7).** Starting with Boc-C(Z)-OH (**2**) (314 mg, 0.62 mmol), compound **7** was prepared as described for compound **6**, except for the workup. Following overnight reaction, the cloudy solution was diluted with H_2O (16 mL) and acidified to pH 2 with 12 N HCl. The precipitate that formed immediately was collected by filtration, washed with Et_2O, and dried in vacuo over P_2O_5 to yield a white solid, **7** (257 mg, 84%): mp 201–202 °C; ^1H NMR (DMSO-d_6) δ (two rotamers) 9.24 (m, 0.6H), 9.15 (m, 0.4H), 7.90 (d, J = 7.5 Hz, 0.6H), 7.87 (d, J = 7.5 Hz, 0.4H), 7.41–7.31 (m, 5H), 6.99 (d, J = 7.5 Hz, 0.6H), 6.98 (d, J = 7.5 Hz, 0.4H), 5.17 (s, 2H), 4.81 (s, 1.2H), 4.60 (s, 0.8H), 4.38 (q, J = 7.0 Hz, 1.2H), 4.35 (q, J = 7.0 Hz, 0.8H), 4.01 (s, 0.8H), 3.94 (s, 1.2H), 3.63–3.24 (m, 4H), 1.22 (t, J = 7.0 Hz, 1.8H), 1.20 (t, J = 7.0 Hz, 1.2H); ^{13}C NMR (DMSO-d_6) δ[25] (190.5, 190.1), (168.1, 168.0), (167.3, 167.2), (163.5, 163.4), (155.5, 155.4), 153.6, 151.4, 136.4, 128.9, 128.6, 128.4, 94.3, 66.9, (65.9, 65.7), (51.5, 48.7), 50.0, (46.4, 45.8), 42.5, 14.7; FABMS m/z 492.3 $[M + H]^+$, 514.2 $[M + Na]^+$, 490.2 $[M - H]^-$. Anal. Calcd for $C_{21}H_{25}N_5O_7S{\cdot}H_2O$: C, 49.50; H, 5.34; N, 13.74; S, 6.29. Found: C, 49.60; H, 5.56; N, 13.94; S, 6.20.

***N*-[2-(*N*-Ethoxythiocarbonyl)aminoethyl]-*N*-[[2-*N*-(benzyloxycarbonyl)guanin-9-yl]acetyl]glycine (8).** Starting with Boc-G(Z)-OH (**3**) (402 mg, 0.74 mmol), compound **8** was prepared as described for compound **6**, except for workup. Following overnight reaction, the cloudy solution obtained was diluted with H_2O (18 mL), and a white precipitate formed. The resulting suspension was acidified to pH 2 with 12 N HCl, whereupon the solid redissolved. EtOAc (20 mL) was added to the solution, and a white precipitate formed that was collected by filtration. The aqueous phase was extracted further with EtOAc (3 × 20 mL), and the organic layers were combined, dried ($MgSO_4$), and concentrated under reduced pressure. The white solids were combined, washed with Et_2O, and dried in vacuo over P_2O_5 to yield 361 mg of **8** (92%): mp 184–186 °C; ^1H NMR (DMSO-d_6) δ 11.67 (s, 0.45H), 11.55 (s, 0.55H), 11.36 (s, 0.45H), 11.35 (s, 0.55H), 9.22 (m, 0.55H), 9.00 (m, 0.45H), 7.81 (s, 0.55H), 7.79 (s, 0.45H), 7.43–7.34 (m, 5H), 5.24 (s, 2H), 5.11 (s, 0.9H), 4.92 (s, 1.1H), 4.43–4.31 (m, 2H), 4.33 (s, 0.9H), 4.01 (s, 1.1H), 3.68–3.37 (m, 4H), 1.25–1.18 (m, 3H); ^{13}C NMR (DMSO-d_6) δ[25] (190.2, 189.9), (170.9, 170.4), (167.2, 167.1), 150.2, (154.6, 154.5), (149.6, 149.5), 147.2, 140.5, (135.5, 135.4), 128.5, 128.3, (128.2, 128.1), 119.2, (67.3, 67.2), (65.6, 65.4), (49.2, 47.8), (45.7, 45.4), (44.0, 43.9), (42.4, 41.9), (14.3, 14.1); FABMS m/z 532.2 $[M + H]^+$, 530.2 $[M - H]^-$. Anal. Calcd for $C_{22}H_{25}N_7O_7S{\cdot}H_2O$: C, 48.08; H, 4.91; N, 17.84; S, 5.83. Found: C, 47.96; H, 4.87; N, 17.65; S, 6.12.

***N*-[2-(*N*-Ethoxythiocarbonyl)aminoethyl]-*N*-[(thymin-1-yl)acetyl]glycine (9).** Starting with Boc-T-OH (**4**) (1.15 g, 3 mmol), compound **9** was prepared as described for compound **6**, except that less of bis(ethoxythiocarbonyl) sulfide (**5**) was used (0.76 g, 3.6 mmol, 1.2 equiv) and the workup was different. Following overnight stirring, the solution was diluted with water (50 mL) and washed with CH_2Cl_2 (2 × 50 mL). The aqueous phase was acidified to pH 2 with 12 N HCl and extracted with EtOAc (3 × 80 mL); the phase separations were slow. The combined organic phases were dried ($MgSO_4$) and concentrated to dryness in vacuo to provide **9** as a colorless foam, which was crystallized from EtOAc–hexane to provide, after drying in vacuo, white crystals (1.07 g, 96%): mp 98–100 °C; ^1H NMR (DMSO-d_6) δ 11.29 (s, 1H), 9.13 (m, 0.6H), 9.01 (m, 0.4H), 7.29 (s, 0.6H), 7.25 (s, 0.4H), 4.65 (s, 0.6H), 4.45 (s, 0.4H), 4.41–4.33 (m, 2H), 4.21 (s, 0.4H), 3.99 (s, 0.6H), 3.62–3.26 (m, 4H), 1.73 (s, 3H), 1.23 (m, 3H); ^{13}C NMR (DMSO-d_6) δ[25] (190.2, 189.9), (170.7, 170.4), (167.7, 167.2), 164.4, 150.9, 142.0, (108.2, 108.1), (65.6, 65.3), (49.0, 46.4), (47.7, 47.6), (45.6, 45.2), (42.2, 41.9), (14.3, 14.2), (12.0, 11.9); FABMS m/z 373.3 $[M + H]^+$, 371.3 $[M - H]^-$. Anal. Calcd for $C_{14}H_{20}N_4O_6S{\cdot}H_2O$: C, 43.07; H, 5.67; N, 14.35; S, 8.21. Found: C, 43.24; H, 5.10; N, 13.53; S, 8.09.

***N*-[2-(*N*-Dithiasuccinoyl)aminoethyl]-*N*-[[6-*N*-(benzyloxycarbonyl) adenin-9-yl]acetyl]glycine (11).** BSA (59 μL, 0.24 mmol, 1.5 equiv) was added to a suspension of freshly prepared **6** (81 mg, 0.16 mmol) in CH_3CN (9 mL) and the mixture stirred for 30 min. Next, Cl(C=O)SCl (**10**) (14 μL, 0.18 mmol, 1.1 equiv) was added, resulting in a yellow solution; the reaction was complete after 20 min as indicated by HPLC. The suspension was distributed between EtOAc (10 mL) and H_2O (15 mL), and the aqueous phase was extracted with EtOAc (5 × 15 mL). The organic layers were combined, dried ($MgSO_4$), and concentrated to a small volume that upon cooling to −20 °C gave **11** as an off white solid (70 mg, 80%): mp 138–140 °C; ^1H NMR (DMSO-d_6) δ (two rotamers) 8.59 (s, 1H), 8.39 (s, 0.3H), 8.27 (s, 0.7H), 7.45 (d, J = 7.0 Hz, 2H), 7.39 (d, J = 7.0 Hz, 2H), 7.33 (t, J = 7.0 Hz, 1H), 5.29 (s, 0.6H), 5.21 (s, 2H), 5.10 (s, 1.4H), 4.32 (s, 1.4H), 4.01 (t, J = 6.8 Hz, 0.6H), 3.99 (s, 0.6H), 3.81 (t, J = 5.3 Hz, 1.4H), 3.73 (t, J = 6.8 Hz, 0.6H), 3.53 (t, J = 5.3 Hz, 1.4H); ^{13}C NMR (DMSO-d_6) δ[25] (170.9, 170.6), (169.6, 169.2), 167.9, 166.9, 152.7, 152.6, 151.9, 149.6, (145.6, 145.5), 136.8, 128.9, 128.4, 128.3, 122.9, 66.7, (48.6, 48.2), (44.8, 44.4), (44.3, 44.2), (43.3, 42.7); FABMS m/z 546.2 $[M + H]^+$, 544.0 $[M - H]^-$. Anal. Calcd for $C_{21}H_{19}N_7O_7S_2{\cdot}{}^3/_2H_2O$: C, 44.05; H, 3.87; N, 17.12; S, 11.19. Found: C, 43.76; H, 3.46; N, 16.80; S, 10.73.

***N*-[2-(*N*-Dithiasuccinoyl)aminoethyl]-*N*-[[4-*N*-(benzyloxycarbonyl)cytosin-1-yl]acetyl]glycine (12).** Starting with **7** (80 mg, 0.16 mmol), compound **12** was prepared as described for compound **11**, except the workup and that heterocyclization with Cl(C=O)SCl (**10**) (16 μL, 0.19 mmol, 1.2 equiv) was performed at 0 °C. The reaction was complete after 20 min as indicated by HPLC. Next, the solution was distributed between EtOAc (10 mL) and H_2O (15 mL), and the aqueous phase was extracted with EtOAc (5 × 15 mL). The combined organic phases were concentrated to a small volume that upon cooling to −20 °C gave **12** as a white solid (70 mg, 84%): mp 198–200 °C; ^1H NMR (DMF-d_7) δ (two rotamers) 8.10 (d, J = 12.0 Hz, 0.4H), 7.91 (d, J = 12.5 Hz, 0.6H), 7.50–7.36 (m, 5H), 7.15 (d, J = 12.0 Hz, 0.4H), 7.14 (d, J = 12.5 Hz, 0.6H), 5.25 (s, 2H), 4.98 (s, 0.8H), 4.81 (s, 1.2H), 4.41 (s, 1.2H), 4.18 (s, 0.8H), 4.15 (t, J = 12.0 Hz, 0.8H), 3.98 (t, J = 9.5 Hz, 1.2H), 3.81 (t, J = 12.0 Hz, 0.8H), 3.71 (t, J = 9.5 Hz, 1.2H); ^{13}C NMR (DMF-d_7) δ[25] (170.9, 170.7), (169.4, 169.2), 168.7, 167.8, (163.8, 163.6), (155.5, 155.3), 153.9, (151.2, 150.9), 136.7, 128.8, 128.5, 128.3, 94.2, 67.1, (49.9, 49.7), (48.7, 48.1), (44.8, 44.5), (43.2, 42.9); FABMS m/z 522.2 $[M + H]^+$, 520.1 $[M - H]^-$. Anal. Calcd for $C_{20}H_{19}N_5O_8S_2$: C, 46.06; H, 3.65; N, 13.43; S, 12.28. Found: C, 45.82; H, 3.90; N, 13.23; S,12.04.

The synthesis of Dts-C(Z)-OH (**12**) was also carried out at 25 °C with a range of excesses of Cl(C=O)SCl (**10**) (1–2.2 equiv). HPLC analysis of the crude reaction mixtures showed **12** as the major product, but a significant amount (17–27%) of 1,2,4-thiadiazolidine-3,5-dione **15** was also detected. Compound **15** was characterized by LC/MS analysis of the crude mixtures: ESMS (m/z) calcd for $C_{38}H_{38}N_{10}O_{14}S$ 890.3, found 890.5 $[M + H]^+$, 446.0 $[M + 2H]^{2+}$.

***N*-[2-(*N*-Dithiasuccinoyl)aminoethyl]-*N*-[[2-*N*-(benzyloxycarbonyl) guanin-9-yl]acetyl]glycine (13).** Starting with **8** (80 mg, 0.15 mmol), compound **13** was prepared as described above for compound **11**, except for workup. The reaction was complete after 30 min as indicated by HPLC. The solution was distributed between EtOAc (10 mL) and H_2O (15

(25) Each carbon has two resonances, as confirmed by HMQC.

mL), and the aqueous phase was extracted with EtOAc (5 × 15 mL). The combined organic phases were concentrated to a small volume, which upon cooling to −20 °C gave **13** as an off-white solid (71 mg, 85%): mp 158−160 °C; ^{1}H NMR (DMF-d_7) δ (two rotamers) 11.75 (s, 0.7H), 11.56 (s, 0.3H), 11.43 (s, 1H), 7.96 (s, 0.3H), 7.81 (s, 0.7H), 7.54−7.38 (m, 5H), 5.35 (s, 1.4H), 5.33 (s, 0.6H), 5.31 (s, 0.6H), 5.06 (s, 1.4H), 4.51 (s, 1.4H), 4.23 (t, J = 7.5 Hz, 0.6H), 4.19 (s, 0.6H), 3.99 (t, J = 5.5 Hz, 1.4H), 3.89 (t, J = 7.5 Hz, 0.6H), 3.73 (t, J = 5.5 Hz, 1.4H); ^{13}C NMR (DMF-d_7) δ[25] (171.0, 170.6), 169.3, 168.3, 167.2, 155.5, (155.3, 155.2), 150.0, 148.4, (140.8, 140.5), (136.2, 136.1), 128.9, (128.7, 128.6), (128.5, 128.3), 118.7, (67.9, 67.8), (49.0, 48.7), (45.2, 44.7), 44.3, (43.4, 43.0); FABMS *m/z* 562.1 [M + H]$^+$, 560.1 [M − H]$^-$. Anal. Calcd for $C_{21}H_{19}N_7O_8S_2$·2H_2O: C, 42.17; H, 3.85; N, 16.41; S,10.73. Found: C, 42.04; H, 3.99; N, 15.73; S, 9.95.

***N*-[2-(*N*-Dithiasuccinoyl)aminoethyl]-*N*-[(thymin-1-yl)-acetyl]glycine (14). Method A.** BSA (136 μL, 0.55 mmol, 1.1 equiv) was added to a solution of **9** (186 mg, 0.5 mmol) in CH_3CN (5 mL), and the mixture was stirred for 30 min. Next, Cl(C═O)SCl (**10**) (45 μL, 0.55 mmol, 1.1 equiv) was added, resulting in a yellow solution. After 2 h, the suspension was distributed between EtOAc (20 mL) and water (5 mL), and the aqueous phase was extracted with EtOAc (4 × 20 mL). The organic layers were combined, dried ($MgSO_4$), and concentrated to a small volume that upon cooling to −20 °C gave **14** as an off-white solid (161 mg, 80%). **Method B (Preferred on Laboratory Scale).** The resultant foam from the synthesis of Etc-T-OH (**9**) (1.21 g, 3.0 mmol) was taken up directly in CH_3CN (15 mL), and BSA (0.81 mL, 3.3 mmol, 1.1 equiv) was added. After 30 min, Cl(C═O)SCl (**10**) (0.27 mL, 3.3 mmol, 1.1 equiv) was added, and the solution turned yellow. After a further 2 h, a white precipitate was observed, and HPLC analysis indicated completion of the reaction. The suspension was distributed between EtOAc (80 mL) and water (30 mL), and the aqueous phase was extracted with EtOAc (4 × 80 mL). The organic layers were combined, dried ($MgSO_4$), and concentrated to a small volume that upon cooling to −20 °C gave **14** as an off-white solid (0.91 g, 76% from **4**) (sometimes, a yellow solid was obtained, but it could be decolorized by suspending in diethyl ether, followed by overnight stirring and then filtration): mp 158−160 °C; ^{1}H NMR (DMSO-d_6) δ (two rotamers) 11.29 (s, 0.4H), 11.27 (s, 0.6H), 7.34 (s, 0.4H), 7.12 (s, 0.6H), 4.59 (s, 0.8H), 4.42 (s, 1.2H), 4.19 (s, 1.2H), 3.97 (s, 0.8H), 3.91 (t, J = 6.8 Hz, 0.8H), 3.79 (t, J = 5.5 Hz, 1.2H), 3.57 (t, J = 6.8 Hz, 0.8H), 3.50 (t, J = 5.5 Hz, 1.2H), 1.74 (s, 3H); ^{13}C NMR (DMSO-d_6) δ[25] (170.9, 170.7), (169.4, 169.2), 168.7, 167.7, (164.9, 164.8), (151.4, 151.3), (142.5, 142.1), (108.7, 108.6), (48.6, 48.1), (47.9, 47.8), (44.5, 44.3), (43.1, 42.8), 14.5; FABMS *m/z* 403.1 [M + H]$^+$, 402.2 [M$^·$]$^-$. Anal. Calcd for $C_{13}H_{14}N_4O_7S_2$: C, 38.80; H, 3.48; N, 13.93; S, 15.92. Found: C, 38.90; H, 3.70; N, 13.61; S, 16.16.

Solid-Phase Deprotection Studies. Fmoc-PAL-PEG-PS resin (0.44 g, 0.18 mmol/g) was washed with NMP (3 × 2 min) and CH_2Cl_2 (3 × 2 min), treated with piperidine−NMP (3:7, 2 + 8 min), and finally washed with NMP (5 × 2 min). Fmoc-Gly-OH (119 mg, 5 equiv) was coupled to the resin with HBTU (152 mg, 5 equiv) and DIEA (70 μL, 5 equiv) in NMP for 3 h. After washes with NMP (5 × 2 min) and CH_2Cl_2 (5 × 2 min), the resin was acetylated with Ac_2O−pyridine−CH_2Cl_2 (4 mL, 1:9:9, 2 + 30 min) and washed with CH_2Cl_2 (5 × 2 min) and NMP (3 × 2 min). Following Fmoc removal and washes described above, Dts-T-OH (95 mg, 3 equiv) was incorporated onto the resin with HBTU (91 mg, 3 equiv) and DIEA (14 μL, 1 equiv) in NMP at 25 °C for 3 h (Kaiser ninhydrin test[26] negative after this time). The resin was washed with NMP (3 × 2 min) and CH_2Cl_2 (3 × 2 min), and dried in vacuo.

Portions of this resin (10−15 mg per experiment) were subjected to various Dts deprotection conditions, as follows: Dts-T-Gly-PAL-PEG-PS resin was swollen in CH_2Cl_2 (2 × 5 min), treated with a freshly prepared solution of the thiol, together with any additive, for a given amount of time (Tables 1 and 2), and washed with CH_2Cl_2 (8 × 1 min). The resin was cleaved with TFA−H_2O (19:1) for 2 h, and the filtrates were expressed from the vessels with positive nitrogen pressure. The cleaved resin was washed with TFA−H_2O (19:1) (2 × 0.5 mL), and the filtrates were combined and evaporated to dryness. The resulting residue was dissolved in water and analyzed by analytical HPLC (conditions at the beginning of this Experimental Section) and studied further by mass spectrometry.

Dts-T-Gly-NH_2 was characterized by mass spectrometry and ^{1}H NMR prior to the deprotection studies: FABMS calcd for $C_{15}H_{18}N_6O_7S_2$ 458.4, found *m/z* 459.1 [M + H]$^+$, 458.1 [M$^{·-}$]; ^{1}H NMR (DMF-d_7) δ (two rotamers) 11.28 (s, 0.3H), 11.22 (s, 0.7H), 8.45 (m, 0.7 H), 8.22 (m, 0.3H), 7.50 (bs, 1H), 7.42 (s, 0.3 H), 7.24 (s, 0.7H), 7.08 (bs, 1H), 4.77 (s, 0.6H), 4.63 (s, 1.4H), 4.29 (s, 1.4H), 4.11 (s, 0.6H), 4.08 (m, 0.6H), 3.93 (t, J = 6.0 Hz, 1.4H), 3.86 (d, J = 6.0 Hz, 1.4H), 3.76 (d, J = 6.0 Hz, 0.6H), 3.77 (m, 0.6H), 3.67 (t, J = 6.0 Hz, 1.4H), 1.78 (s, 3H).

H-T-Gly-NH_2 was characterized by mass spectrometry and co-injected with a sample prepared by an Fmoc-strategy: FABMS calcd for $C_{13}H_{20}N_6O_5$ 340.4, found *m/z* 341.2 [M + H]$^+$, 363.2 [M + Na]$^+$.

When deprotection was carried out with *p*-methoxybenzylmercaptan (**16**), benzylmercaptan (**18**), *p*-methylthiophenol (**22**), thiophenol (**23**), and *p*-chlorothiophenol (**24**), relatively low but nonzero levels of thiourethane formation were observed (Scheme 5, Table 1, entries 1−3, 7, and 11−13). Thiourethane corresponding to thiol **16**: FABMS calcd for $C_{22}H_{28}N_6O_7S$ 520.5, found *m/z* 521.2 [M + H]$^+$, 520.3 [M$^·$]$^-$, 633.2 [M − H + TFA]$^-$. Thiourethane corresponding to thiol **18**: FABMS calcd for $C_{21}H_{26}N_6O_6S$ 490.5, found *m/z* 491.2 [M + H]$^+$, 513.2 [M + Na]$^+$, 489.0 [M − H]$^-$, 603.2 [M − H + TFA]$^-$. Thiourethane corresponding to thiol **22**: ESMS calcd for $C_{21}H_{26}N_6O_6S$ 490.5, found *m/z* 491.0 [M + H]$^+$. Thiourethane corresponding to thiol **23**: ESMS calcd for $C_{20}H_{24}N_6O_6S$ 476.5, found *m/z* 476.8 [M + H]$^+$. Thiourethane corresponding to thiol **24**: ESMS calcd for $C_{20}H_{23}ClN_6O_6S$ 510.9, found *m/z* 511.0 [M + H]$^+$.

The carbamoyl disulfide intermediate **25** was observed in some experiments (e.g., Table 1, entry 9) using βME for deprotection; it was characterized by mass spectrometry: ESMS calcd for $C_{16}H_{24}N_6O_7S_2$ 476.5, found *m/z* 477.0 [M + H]$^+$, 499.0 [M + Na]$^+$.

The urea derivative **26** was also noted during the deprotection reactions (Tables 1 and 2) and characterized by mass spectrometry: FABMS calcd for $C_{27}H_{38}N_{12}O_{11}$ 706.6, found *m/z* 707.1 [M + H]$^+$, 705.1 [M − H]$^-$.

Solid-Phase Deprotection Studies on Resin with Reduced Loading. Fmoc-PAL-PEG-PS resin (208 mg, 0.18 mmol/g) was treated with piperidine−NMP (3:7, 2 + 8 min), and after washings with NMP (5 × 2 min), Fmoc-Gly-OH (8 mg, 0.7 equiv) was coupled to the resin using HBTU (10 mg, 0.7 equiv) and DIEA (4.5 μL, 0.7 equiv) in NMP for 1 h. The resin was washed with NMP (5 × 1 min) and CH_2Cl_2 (5 × 1 min), acetylated with Ac_2O−pyridine−CH_2Cl_2 (1:9:9, 2 + 30 min), washed with CH_2Cl_2 (5 × 2 min), and dried in vacuo. A sample of the dried resin (6 mg) was treated with piperidine−NMP (3:7, 2 + 8 min) and analyzed on the basis of UV (301 nm, $\epsilon = 7800\ cm^{-1}\ M^{-1}$)[27] absorbance of the resultant piperidine−dibenzofulvene adduct. A second sample (8 mg) of resin was similarly deprotected, hydrolyzed, and evaluated by amino acid analysis. These complementary methods showed a final loading of 0.09 mmol/g. Dts-T-OH (23 mg, 3 equiv) was coupled onto the resin with HBTU (21 mg, 3 equiv) and DIEA (3 μL, 1 equiv) in NMP at 25 °C for 3 h (Kaiser ninhydrin test[26] negative after this time). The resin was washed with NMP (5 × 1 min) and CH_2Cl_2 (5 × 1 min), and dried in vacuo. Aliquots of this resin were taken to test Dts deprotection, which were carried out as outlined in the preceding paragraph.

(26) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. *Anal. Biochem.* **1970**, *34*, 595−598.

(27) Grant, G. A. In *Synthetic Peptides: A User's Guide*; Grant, G. A., Ed.; W. H. Freeman and Co.: New York, 1992; p 119.

Evaluation of Conditions for Solid-Phase Synthesis of Dts-T_4-Gly-NH_2. Fmoc-Gly-PAL-PEG-PS resin (40 mg, 0.18 mmol/g) was treated with piperidine−NMP (3:7, 2 + 8 min) to remove Fmoc, washed with NMP (5 × 2 min), and used for evaluation of coupling procedures for introduction of Dts-T-OH (Table 3). Upon completion of coupling (3 h), the resin was washed with NMP (3 × 2 min) and CH_2Cl_2 (3 × 2 min). Removal of the Dts protecting group was accomplished using the conditions shown (Table 3). After washes with CH_2Cl_2 (3 × 1 min), neutralization was achieved with DIEA−CH_2Cl_2 (1:19, 1 + 2 min), followed by washing with CH_2Cl_2 (3 × 1 min) and NMP (3 × 1 min). The coupling/deprotection cycle was repeated for the remaining Dts-T-OH monomers, followed by final washes with NMP (3 × 1 min) and CH_2Cl_2 (3 × 1 min) and drying the resin in vacuo. The resin was treated with TFA−H_2O (19:1) for 2 h, filtered, and washed (2 × 1 mL) with TFA−H_2O (19:1). The combined filtrates were evaporated to dryness under a stream of N_2, and the resulting residue was dissolved in H_2O, lyophilized, and analyzed by analytical HPLC.

Dts-T_4-Gly-NH_2. Manual chain assembly was carried out starting with Fmoc-Gly-PAL-PEG-PS (40 mg, 0.18 mmol/g). The resin was treated with piperidine−NMP (3:7, 2 + 8 min) and washed with NMP (5 × 2 min). Dts-T-OH monomers (9 mg, 3 equiv) were coupled to the support using HBTU (3 mg, 3 equiv) and DIEA (1.2 μL, 1 equiv) in NMP at 25 °C for 3 h. After washings with NMP (3 × 1 min) and CH_2Cl_2 (3 × 1 min), the Dts protecting group was cleaved with DTT (0.5 M) in HOAc (0.5 M)−CH_2Cl_2 (2 + 8 min). The resin was washed with CH_2Cl_2 (5 × 1 min), neutralized with DIEA−CH_2Cl_2 (1:19, 1 + 2 min), and washed with CH_2Cl_2 (3 × 1 min) and NMP (3 × 1 min). Further aspects to assemble, cleave, and evaluate the Dts-protected PNA tetramers were as described in the previous "Evaluation of Conditions". The crude product was analyzed by analytical HPLC (t_R 9.06 min, 80% purity, 88% cleavage yield), and the final product was confirmed by FABMS (matrix: glycerol−TFA): calcd for $C_{48}H_{60}N_{18}O_{19}S_2$ 1257.2, found m/z 1257.5 $[M]^+$.

Dts-G(Z)-G(Z)-T-A(Z)-Gly-NH_2. Manual chain assembly was carried out exactly as described for Dts-T_4-Gly-NH_2. The crude product was analyzed by analytical HPLC (t_R 16.12 min, 54% purity, 88% cleavage yield) and final product confirmed by FABMS (matrix: glycerol−TFA): calcd for $C_{72}H_{75}N_{27}O_{21}S_2$ 1718.6, found m/z 1718.2 $[M]^+$.

Dts-A(Z)-T-C(Z)-G(Z)-Gly-NH_2. Using the same procedures, the crude product was analyzed by analytical HPLC (t_R 16.53 min, 54% purity, 83% cleavage yield) and final product confirmed by FABMS (matrix: glycerol−TFA) calcd for $C_{71}H_{75}N_{27}O_{21}S_2$ 1678.6, found m/z 1678.02 $[M]^+$.

Dts-G(Z)-C(Z)-A(Z)-T-Gly-NH_2. Using the same procedures, the crude product was analyzed by analytical HPLC (t_R 16.67 min, 62% purity, 90% cleavage yield) and final product confirmed by FABMS (matrix: glycerol−TFA): calcd for $C_{71}H_{75}N_{27}O_{21}S_2$ 1678.6, found m/z 1678.24 $[M]^+$.

Acknowledgment. M.P. thanks NATO for a postdoctoral fellowship, E.B. acknowledges a fellowship from CIRIT-DGU and QFN94-4620 for financial support, and K.J. thanks the Alfred Benzon Foundation for a postdoctoral fellowship. We are grateful to NIH and NIST for financial support.

Supporting Information Available: Tables 1−10, which are solid-phase deprotection studies of Dts-Gly-PAL-PEG-PS with the thiols mentioned in the text. ^{1}H NMR and NOESY spectra of compounds **4** and **14** and NOESY spectra of compounds **1** and **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO9824394