

# Alternative Procedures for the Synthesis of Methionine-Containing Peptide–Oligonucleotide Hybrids

Vicente Marchán,<sup>[a]</sup> Chryslaine Rodríguez-Tanty,<sup>[b]</sup> Marta Estrada,<sup>[a]</sup> Enrique Pedroso,<sup>[a]</sup> and Anna Grandas\*<sup>[a]</sup>

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The synthesis of methionine-containing peptide–oligonucleotide hybrids has been found to be best accomplished by a stepwise solid-phase approach in which peptide assembly using the sulfoxide derivative of methionine is followed by elongation of the oligonucleotide chain using the phosphite triester methodology, ammonia deprotection, and reduction of the sulfoxide to thioether by reaction with *N*-

methylmercaptoacetamide. Quantitative amino acid incorporation yields could not always be achieved when the order of assembly of the two moieties was reversed, i.e. by elongating the peptide chain on a resin-linked oligonucleotide in order to avoid exposure of the thioether function to oxidizing conditions.

## Introduction

Many research groups have prepared various types of peptide–oligonucleotide hybrids, usually with the aim of evaluating their possible therapeutic applications (for recent examples, see refs.<sup>[1–16]</sup>). We have also used such hybrids as models for studying the mode of action of the anticancer drug cisplatin.<sup>[17]</sup> Among the questions that we would like to address is whether coordination to two adjacent guanine bases in DNA is preceded by coordination to the side chains of amino acids, and how the metal might then be transferred from proteins to DNA. Since the thioether group and the imidazole ring of the methionine and histidine residues, respectively, would seem to represent some of the most likely platinum anchoring points, we wish to report here on the preparation of peptide–oligonucleotide hybrids containing methionine, histidine, or both, with particular emphasis on the problems associated with the introduction of the oxidizable amino acid methionine.

## Results and Discussion

Based on our previous experience in the preparation of nucleopeptides,<sup>[6]</sup> we first decided to synthesize hybrids with a covalent phosphodiester bond between the side-chain hydroxyl group of an amino acid and the 5'- or 3'-hydroxyl group of a nucleoside. Histidine has already been successfully incorporated into nucleopeptides,<sup>[17,18]</sup> thus it re-

mained to find a suitable synthetic protocol for the methionine-containing conjugates.

Nucleopeptides are commonly obtained in our laboratory using a stepwise solid-phase approach<sup>[6]</sup> in which the assembly of the peptide moiety on a solid support is followed by elongation of the oligonucleotide chain at the free hydroxyl group of an amino acid side chain (serine, threonine, tyrosine, or homoserine) using phosphoramidite building blocks. To prevent oxidation of the methionine thioether to sulfoxide during the phosphite to phosphate oxidation step, we decided to assemble our first synthetic target, H-Ser(p<sup>5'</sup>dG)-Met-OH (**1a**), by coupling a 2'-deoxyguanosine phosphate derivative to the dipeptide–resin. The synthetic scheme is outlined in Figure 1.

The MSNT-mediated<sup>[19,20]</sup> coupling of the 2-chlorophenyl phosphate of 5'-*O*-[3'-*O*-dimethoxytrityl-(2*N*-dimethylaminomethylene-2'-deoxyguanosine)]<sup>[21]</sup> to Fmoc-Ser-Met-*O*-HMFS-Leu-resin<sup>[6]</sup> was followed by removal of the DMT group (an incorporation yield of 86% was established through quantitative determination of the DMT cations formed upon acid treatment). Cleavage of the peptide–resin bond and removal of the Fmoc and 2-chlorophenyl groups was accomplished by reaction with TBAF, and then the guanine protecting group was removed by treatment with ammonia. The only product isolated after MPLC purification was the 5'-phosphate of 2'-deoxyguanosine, formation of which may only be explained by assuming that a nucleoside–serine bond had been formed and subsequently cleaved by  $\beta$ -elimination during the deprotection step. Replacement of the TBAF treatment by reaction with either 0.03 M TBAF in THF/H<sub>2</sub>O/py (8:8:1; 7 h, room temp.) or 0.1 M tetramethylguanidium *syn*-2-nitrobenzaldoximate in dioxane/H<sub>2</sub>O (1:1; 8 h, room temp.) led to similar results. The serine–nucleoside phosphodiester linkage thus appears to be particularly labile in this mole-

<sup>[a]</sup> Departament de Química Orgànica, Facultat de Química, Universitat de Barcelona, Martí i Franquès 1–11, E-08028 Barcelona, Spain  
Fax: (internat.) + 34-93/339-7878  
E-mail: grandas@qo.ub.es

<sup>[b]</sup> Present address: Centro Nacional de Investigaciones Científicas, Avenida 25 no. 15202, Playa 12100, Cubanacán, 6990 La Habana, Cuba

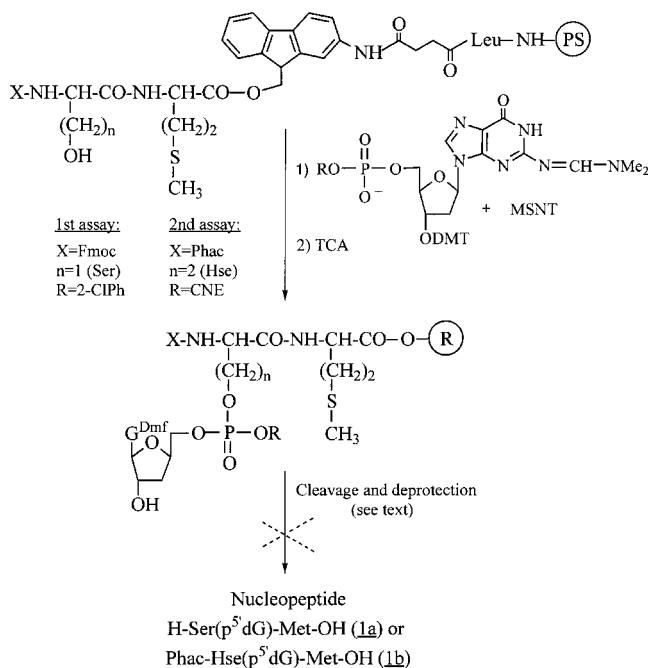


Figure 1. Assays carried out to optimize the synthesis of nucleopeptides **1a** and **1b**

cule, presumably because of the presence of the neighbouring methionine residue.

In a second attempt to obtain a methionine-deoxyguanosine hybrid (**1b**), the serine linking residue was replaced by homoserine, which forms base-stable amino acid–nucleoside phosphodiester bonds.<sup>[21]</sup> Moreover, to reduce the final deprotection step to a single treatment with ammonia/dioxane, the 2-cyanoethyl phosphate of 5'-O-[3'-O-dimethoxytrityl-(2*N*-dimethylaminomethylene-2'-deoxyguanosine)] was synthesized and coupled to Phac-Hse-Met-O-HMFS-Leu-resin (Figure 1). Since the HPLC profile of the crude material obtained after removal of the DMT group and ammonia deprotection was very complex, we decided to abandon this synthetic scheme.

Still with the idea of not submitting the methionine residue to oxidation steps, our second choice was to synthesize Phac-Met-linker-dG (**2**) by reversing the order in which the hybrid molecule was assembled, i.e. anchoring the 2'-deoxyguanosine moiety on the solid support and then coupling a

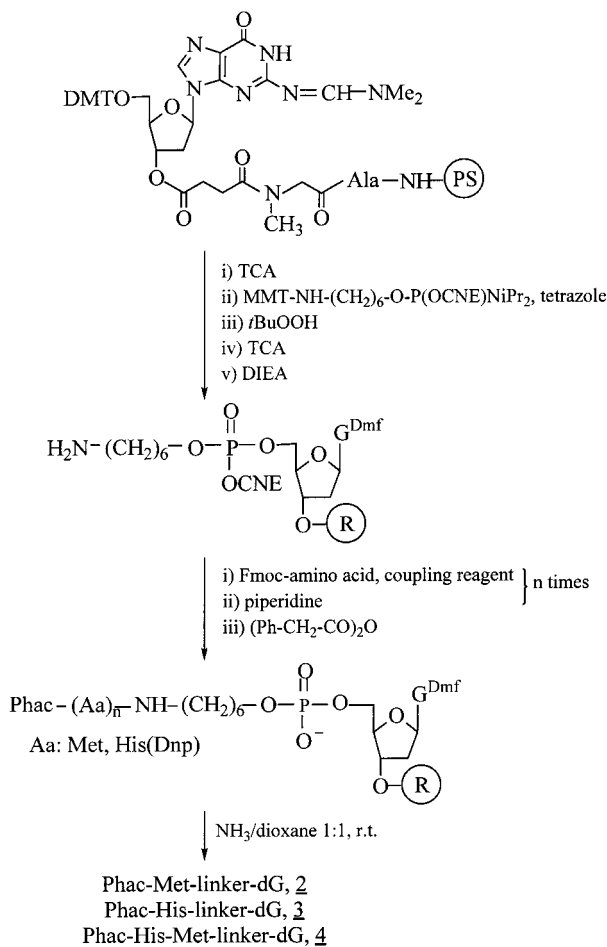


Figure 2. Synthesis scheme used for the preparation of hybrids **2**, **3**, and **4**; for the synthesis of **5** (Phac-His-Gly-Met-linker-dCATGGCT), tripeptide Phac-His(Dnp)-Gly-Met-OH was coupled to H<sub>2</sub>N-linker-oligonucleotide-resin

linker (6-amino-1-hexanol) and the desired *N*<sup>α</sup>-Fmoc-amino acid to it. The *N*-terminus was acylated to prevent reaction with platinum(II) complexes in subsequent studies (Figure 2). *N*<sup>α</sup>-Fmoc- rather than *N*<sup>α</sup>-Boc-amino acids were used, so as to avoid exposure of the nucleotide to acidic conditions. A sarcosine residue was introduced between the succinyl linker and the internal reference amino acid to make the nucleoside–resin linkage sufficiently stable to the basic conditions required to remove the Fmoc group.<sup>[7,22]</sup>

Table 1. Assays carried out to determine the best amino acid coupling conditions

| Entry | Coupling conditions <sup>[a]</sup>                                      | Coupling yield <sup>[b]</sup> |
|-------|-------------------------------------------------------------------------|-------------------------------|
| 1     | 15 equiv. Aa + 15 equiv. DCC, DMF, 3 h                                  | 50%                           |
| 2     | 15 equiv. Aa + 15 equiv. DCC + 15 equiv. HOBt, DMF, 3 h                 | 62%                           |
| 3     | 30 equiv. Aa + 15 equiv. DCC, DMF, 3 h                                  | 62%                           |
| 4     | 15 equiv. Aa + 15 equiv. DCC, NMP, 3 h                                  | 42%                           |
| 5     | 15 equiv. Aa + 15 equiv. DCC, 0.5 M LiCl in DMF, 3 h                    | 70%                           |
| 6     | 15 equiv. Aa + 15 equiv. DCC, 0.5 M LiBF <sub>4</sub> in DMF, 3 h       | 54%                           |
| 7     | 15 equiv. Aa + 15 equiv. DCC + 15 equiv. HOBt, 0.5 M LiCl in DMF, 3 h   | quant.                        |
| 8     | 30 equiv. Aa + 15 equiv. DCC, 0.5 M LiCl in DMF, 3 h                    | quant.                        |
| 9     | 15 equiv. Aa + 15 equiv. TBTU + 30 equiv. DIEA, 0.5 M LiCl in DMF, 1 h  | quant.                        |
| 10    | 15 equiv. Aa + 15 equiv. PyBOP + 30 equiv. DIEA, 0.5 M LiCl in DMF, 1 h | quant.                        |

<sup>[a]</sup> Aa = Fmoc-Met-OH. – <sup>[b]</sup> The extent of incorporation was determined from the relative areas of the peaks due to H-linker-dG and H-Met-linker-dG in the HPLC trace of the crude material obtained after ammonia deprotection.

The synthesis proceeded smoothly up until the incorporation of the linker, but problems were encountered beyond removal of the monomethoxytrityl group. We had great difficulties in coupling Fmoc-methionine to the H<sub>2</sub>N-linker-nucleotide-resin, and blocking of the *N*-terminus with Phac<sub>2</sub>O once the Fmoc group had been eliminated was not straightforward either (see below).

The simplest standard activation conditions, even using a significant excess of the amino acid and coupling reagent, were not sufficiently effective for the incorporation of methionine (Table 1, entries 1–3). Changing the solvent<sup>[23]</sup> did not have a significant effect (entry 4), but the coupling yield increased when LiCl<sup>[24]</sup> was added to the reaction mixture (entry 5). LiCl proved to be more effective than LiBF<sub>4</sub> (entry 6). Carrying out the amino acid incorporation in the presence of LiCl, using either standard or strong activating reagents, allowed quantitative coupling of Fmoc-Met-OH (entries 7–10). It has been postulated that the unfavourable folding of resin-linked peptides may hinder the terminal amine groups and render some steps of peptide syntheses particularly troublesome,<sup>[25]</sup> but it is hard to imagine why, in the present case, the amine group of an H<sub>2</sub>N-linker-nucleotide-resin might not be accessible.

Phac-Met-linker-dG (**2**), Phac-His-linker-dG (**3**), and Phac-His-Met-linker-dG (**4**) were finally obtained by coupling the appropriate amino acids [Fmoc-His(Dnp)-OH, Fmoc-Met-OH, or both] using 15 equiv. of both the amino acid and PyBOP and 30 equiv. of DIEA in a 0.5 M solution of LiCl in DMF (1.5–3 h, Ar atmosphere). Blocking of the *N*-termini was effected by reaction with Phac<sub>2</sub>O (15 equiv.), DMAP (15 equiv.), and DIEA (30 equiv.) in a 0.5 M LiCl solution in DMF (45 min., Ar atmosphere).

We also tried to replace the coupling of Fmoc-His(Dnp)-OH and *N*-terminal phenylacetylation by a single coupling with Phac-His(Dnp)-OH to synthesize **3** and **4**, which led to mixtures of L- and D-His-containing products. This result might have been anticipated, considering that an *N*<sup>α</sup>-acyl-amino acid had been activated rather than an *N*<sup>α</sup>-urethane-protected derivative,<sup>[26]</sup> and that histidine is very prone to racemization.<sup>[27]</sup> McMinn and Greenberg<sup>[8]</sup> have suggested the use of strong activating reagents such as PyBOP or HBTU to minimize histidine racemization, and did not observe epimerization even when the imidazole-unprotected *N*<sup>α</sup>-acetylhistidine was activated with HBTU. In our hands, the PyBOP-mediated coupling of Phac-His(Dnp)-OH (with the electron-withdrawing 2,4-dinitrophenyl group on the imidazole ring) afforded 56% of Phac-L-His-L-Met-linker-dG and 44% of Phac-D-His-L-Met-linker-dG, as determined from the relative areas of the two product peaks in the HPLC profile of the crude reaction mixture (Phac-L-His-linker-dG and Phac-D-His-linker-dG were co-eluted). The identities of these two products were verified by unequivocal syntheses of the two diastereomeric hybrids using either Fmoc-L-His(Dnp)-OH or Fmoc-D-His(Trt)-OH.

The use of the *N*<sup>Im</sup>-trityl-protected histidine derivative might be an alternative to imidazole protection with the Dnp group, but, in our hands, only about 10% of the Trt group could be removed after nine treatments with 10%

TCA/DCM in the presence of Et<sub>3</sub>SiH as scavenger, and some cleavage of the glycosidic linkage was also observed under these conditions. Literature reports on the compatibility of the conditions required to deprotect His(Trt) and the stability of the glycosidic linkage are somewhat contradictory.<sup>[3,28]</sup>

Amino acid coupling problems were similarly encountered when we undertook the synthesis of Phac-His-Gly-Met-linker-dCATGGCT (**5**). Only the use of the strongest carboxyl activating agents, such as HATU,<sup>[29]</sup> was found to allow quantitative coupling of the methionine and glycine derivatives, but incorporation of the histidine residue never exceeded 20% yield. The target product (**5**) was finally obtained by HATU-mediated coupling of the tripeptide Phac-His(Dnp)-Gly-Met-OH to the linker-oligonucleotide-resin.<sup>[28]</sup>

For amino acid or peptide couplings to H<sub>2</sub>N-linker-oligonucleotide-resins, some authors have reported low or non-reproducible yields.<sup>[9]</sup> Other authors have not described any particular problems in relation to the stepwise elongation of a peptide on an oligonucleotide–resin using Fmoc-amino acids,<sup>[3]</sup> whereas others have encountered more difficulties in the coupling of the first Fmoc-amino acid than in the subsequent couplings.<sup>[7]</sup> The latter result<sup>[7]</sup> would confirm the hypothesis that coupling yields to phosphate-unprotected oligonucleotide-resins may be superior to those of couplings to phosphate-protected oligonucleotide-resins,<sup>[9]</sup> but we have always had less difficulties in the first amino acid incorporation than in the subsequent ones. The cyanoethyl group is still present on the phosphate(s) in the first amino acid coupling, but it is most probably lost during the piperidine treatments that remove the Fmoc group.

Taken together, the aforementioned findings indicated that it would not be easy to prepare peptide–oligonucleotide hybrids using this approach, hence we turned our attention back to our standard nucleopeptide synthesis scheme.

Methionine is generally incorporated into polymer-supported peptide chains as the sulfoxide so as to avoid both uncontrolled oxidation and, more importantly, alkylation by reaction with carbocations formed under acidic deprotection conditions.<sup>[30]</sup> Therefore, assembly of the nucleopeptide–resin using the methionine sulfoxide derivative seemed the safest option, but some aspects of methionine chemistry had to be considered first.

To evaluate the effect of the phosphite to phosphate oxidizing conditions on a sulfoxide-protected methionine-containing peptide, an aliquot of the peptide–resin Ac-Lys(Tfa)-Met(O)-Tyr-Pro-*O*-HMFS-Phe-resin (see below) was treated with the standard oxidation reagent, aqueous iodine. Not unexpectedly for a tyrosine-containing peptide,<sup>[31]</sup> MALDI-TOF mass spectrometric analysis of the crude peptide obtained after deprotection with conc. aq. NH<sub>3</sub>/dioxane (1:1) (overnight, room temp.) showed the presence of iodinated products. In a further experiment, both HPLC and MALDI-TOF mass spectrometric analysis after ammonia deprotection showed that the peptide had remained intact after treatment with 1 M *t*BuOOH for 1 h,

hence we selected this reagent for the oxidation of phosphites to phosphates.

Sulfoxides have long been known to be capable of oxidizing  $P^{III}$  compounds (phosphanes, phosphite triesters) to  $P^V$  derivatives,<sup>[32]</sup> with their concomitant reduction to thioethers. It was thus conceivable that, during each nucleotide incorporation cycle, some methionine sulfoxides might be reduced, although we could expect these side chains to be re-oxidized by the *t*BuOOH treatment. Even though there seems to be a consensus of opinion that anhydrous *t*BuOOH solutions do not oxidize thioethers to sulfoxides,<sup>[33]</sup> it has been reported that methionines are oxidized to sulfoxides by *t*BuOOH when anchored to a solid matrix.<sup>[34]</sup> Our experience is that, at least in solution, methionine is quickly oxidized by 1 M *t*BuOOH solutions, and thus we expect such oxidation to also take place on the solid support.

Of the many different methods described for the reduction of sulfoxides to thioethers following peptide assembly,<sup>[35–39]</sup> reduction with *N*-methylmercaptoacetamide<sup>[35]</sup> seemed the safest method for the oligonucleotide moiety. In a preliminary assay, oligonucleotide 5'-GCGAT-ATCG3' was treated with a large excess (1000 equivalents, overnight, 37 °C) of *N*-methylmercaptoacetamide in water and was found to remain totally unaffected.

We then undertook the stepwise solid-phase synthesis of a methionine-containing nucleopeptide, specifically Ac-Lys-Met-Tyr( $p^3$ dACTAGT)-Pro-OH (6) (Figure 3).

The tyrosine–nucleoside phosphodiester linkage has previously been shown to be stable to treatment with 1:1  $NH_3$ /dioxane at 55 °C,<sup>[40]</sup> hence the trifluoroacetyl group and the standard benzoyl and isobutyryl groups were chosen for the permanent protection of the lysine side chain and the exocyclic amines of the nucleobases, respectively. Since the tyrosine–nucleoside phosphodiester bond has been reported to be labile in the presence of tetrabutylammonium fluoride,<sup>[41]</sup> the nucleopeptide was linked to the resin through a 9-fluorenylmethyl ester group, which allowed cleavage of the nucleopeptide–HMFS handle<sup>[42]</sup> bond simply by treating with ammonia.<sup>[6]</sup> Ac-Lys(Tfa)-Met(O)-Tyr-Pro-*O*-HMFS-Phe-resin was assembled according to standard procedures,<sup>[6]</sup> with the sole exception that, to prevent loss of the peptide chains through diketopiperazine formation, Boc-Met(O)-OH was coupled to the trifluoroacetate salt of Tyr-Pro-resin by activation with PyAOP in the presence of DIEA.<sup>[43]</sup> The oligonucleotide chain was elongated at the side chain of the tyrosine residue, and the crude nucleopeptide was obtained after deprotection with ammonia and reduction of the sulfoxide with *N*-methylmercaptoacetamide. The target product, 6, was obtained after MPLC purification in 23% yield, which is within the same range as achieved for other nucleopeptides constructed using the same methodology.

In summary, we have prepared nucleopeptides containing a methionine residue for the first time. Sulfoxide protection to prevent alkylation of the methionine side chain has allowed the target molecule to be safely assembled on a solid matrix using the phosphite triester approach and *t*BuOOH

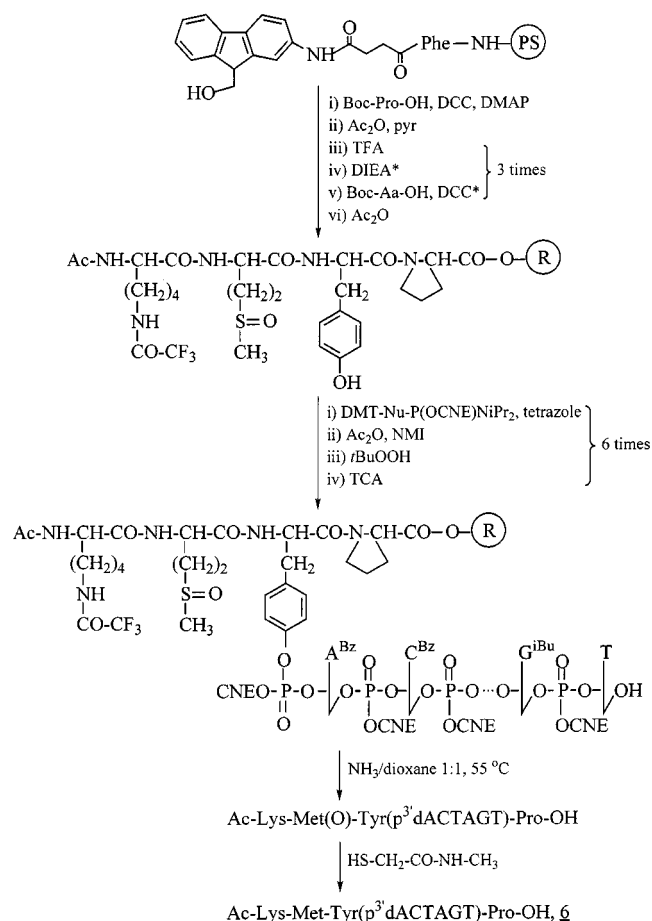


Figure 3. Stepwise solid-phase synthesis of nucleopeptide 6; \* step (iv) was suppressed to incorporate Boc-Met(O)-OH, which was activated with PyAOP and coupled to the trifluoroacetate salt of the dipeptide–resin in the presence of DIEA

oxidation to incorporate each nucleotide unit. In our opinion, this synthetic strategy is more reliable than the methodology in which the peptide is either coupled to or assembled in a stepwise manner on oligonucleotide–resins, since high and reproducible yields are difficult to achieve in these cases.

Work is currently in progress aimed at extending this stepwise solid-phase synthesis methodology to the introduction of other trifunctional amino acids into nucleopeptides or other peptide–oligonucleotide conjugates.

## Experimental Section

**H-Ser( $p^5$ dG)-Met-OH (1a):** Dipeptide–resin Fmoc-Ser-Met-*O*-HMFS-Leu-resin was assembled according to the general procedure described in ref.<sup>[6]</sup> (using the HMFS handle). The 2-chlorophenyl phosphate of 5'-*O*-[3'-*O*-dimethoxytrityl-(2*N*-dimethylaminomethylene-2'-deoxyguanosine)] was obtained as described in ref.<sup>[20]</sup> (pp. 94–96) and coupled to the side chain of serine in the presence of MSNT (molar ratio: nucleotide/MSNT/peptide–resin 8:40:1; pyridine, 5 h, room temp.). The DMT group was subsequently removed by reaction with 3% trichloroacetic acid (86% nucleotide coupling yield). Final deprotection was carried out by treating the nucleopeptide–resin first with 0.05 M TBAF in anhy-

drous THF (30 min., room temp.) and then with conc. aq.  $\text{NH}_3$ /dioxane (1:1; overnight, room temp.).

**Bis(2-cyanoethoxy)(diisopropylamino)phosphane:** This compound was obtained by the reaction of chloro(2-cyanoethoxy)(diisopropylamino)phosphane with dry 2-cyanoethanol and DIEA (molar ratio 1:2:1.5). It was purified by silica gel column chromatography (hexanes/*t*BuOMe, 1:1 and 1:3).

**2-Cyanoethyl Phosphate of 5'-O-[3'-O-dimethoxytrityl-(2*N*-dimethylaminomethylene-2'-deoxyguanosine):** This compound was prepared by reaction of 3'-O-dimethoxytrityl-(2*N*-dimethylaminomethylene-2'-deoxyguanosine) with bis(2-cyanoethoxy)diisopropylaminophosphane and tetrazole followed by: (i) *t*BuOOH oxidation, and (ii) treatment with  $\text{Et}_3\text{N}$ /py (1:1; 3 h, room temp.) to remove one cyanoethyl group.

**Phac-Hse(p<sup>5</sup>dG)-Met-OH (1b):** Nucleopeptide **1b** was assembled essentially as described for **1a**, by coupling the 2-cyanoethyl phosphate of 5'-O-[3'-O-dimethoxytrityl-(2*N*-dimethylaminomethylene-2'-deoxyguanosine)] to the dipeptide–resin Phac-Hse-Met-*O*-HMFS-Leu-resin. Nucleotide coupling yield: 61%. Basic deprotection treatment: conc. aq.  $\text{NH}_3$ /dioxane (1:1; overnight, room temp.).

**Fmoc-His(Dnp)-OH:** The Boc group was removed from commercially available Boc-L-His(Dnp)-OH by reaction with 4 M HCl/dioxane for 1 h at room temp. A yellow solid [HCl·H-His(Dnp)-OH] was obtained after removal of the solvent under reduced pressure. A solution of Fmoc-Cl (1.5 equiv.) in dioxane was then added to a cooled solution of the HCl·H-His(Dnp)-OH thus obtained in 10% aq.  $\text{Na}_2\text{CO}_3$ , and the mixture was stirred overnight in an ice bath and for a further 24 h at room temp. Amino protection was then completed by treatment with an additional 0.5 equiv. of Fmoc-Cl. The dioxane was evaporated under reduced pressure, and the concentrated mixture was poured onto a mixture of ice and  $\text{H}_2\text{O}$  and extracted with diethyl ether. The aqueous layer was cooled, acidified to pH 2 with conc. HCl, and extracted with ethyl acetate. The combined organic extracts were washed with 0.1 M HCl and  $\text{H}_2\text{O}$ , dried over  $\text{MgSO}_4$ , and concentrated. The desired product was obtained following silica gel column chromatography eluting with mixtures of dichloromethane and ethyl acetate (100/0 → 0/100) containing 1% acetic acid; 30% yield. Electrospray-MS (positive mode):  $m/z = 543.8$  [ $\text{M} + \text{H}$ ]<sup>+</sup>; calcd. for  $\text{C}_{27}\text{H}_{21}\text{N}_5\text{O}_8$ : 543.46.

**Phac-His(Dnp)-OH:** This derivative was prepared following essentially the same methodology as described for the synthesis of Fmoc-His(Dnp)-OH, but using the *O*-phenylacetyl derivative of *N*-hydroxysuccinimide (Phac-OSu) to block the amine group. FAB-MS (positive mode):  $m/z = 440.1$  [ $\text{M} + \text{H}$ ]<sup>+</sup>, 462.1 [ $\text{M} + \text{Na}$ ]<sup>+</sup>; calcd. for  $\text{C}_{20}\text{H}_{17}\text{N}_3\text{O}_8$ : 439.36.

**Phac-OSu:** Reaction of equimolar amounts of phenylacetic acid, DCC, and *N*-hydroxysuccinimide in dichloromethane (overnight, room temp.), followed by the addition of diethyl ether to precipitate *N,N'*-dicyclohexylurea and aqueous work-up, afforded the active ester. CI-MS ( $\text{NH}_3$ ):  $m/z = 251$  [ $\text{M} + \text{NH}_4$ ]<sup>+</sup>; calcd. for  $\text{C}_{12}\text{H}_{11}\text{NO}_4$ : 233.21.

**Phac-Met-linker-<sup>5</sup>dG (2), Phac-His-linker-<sup>5</sup>dG (3), and Phac-His-Met-linker-<sup>5</sup>dG (4):** DCC-mediated coupling of Boc-sarcosine and the 3'-*O*-succinate of 5'-*O*-dimethoxytrityl-(2*N*-dimethylaminomethylene)-2'-deoxyguanosine to Fmoc-alanine-resin (0.21 mmol/g) was followed by incorporation of MMT-NH-( $\text{CH}_2$ )<sub>6</sub>-*O*-P(OCNE)NiPr<sub>2</sub> in the presence of tetrazole, capping, and oxidation

with 1 M *t*BuOOH. The MMT group was subsequently removed under standard detritylation conditions and the amine groups on the resin were neutralized by treatment with 2% DIEA in dichloromethane prior to amino acid incorporation. The extent of amino acid incorporation was monitored by HPLC analysis of the crude material obtained following ammonia treatment of an aliquot of amino-acid-linker-nucleotide-resin (A: 0.01 M  $\text{NH}_4\text{OAc}$ , B:  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ , 1:1, linear gradient from 10 to 50% of B over 40 min., 1 mL/min):  $t_R$  dG 7.0 min, H-linker-dG 9.2 min, H-Met-linker-dG 20.1 min, Phac-Met-linker-dG 30.0 min. Once assembled, the target hybrid was obtained after overnight treatment with conc. aq.  $\text{NH}_3$ /dioxane (1:1; room temp.) and purification by MPLC (A: 0.05 M  $\text{NH}_4\text{OAc}$ , B:  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ , 1:1, gradient from 0 to 40% of B for hybrids **2** and **4**, and from 0 to 30% of B for hybrid **3**). **2**, **3**, and **4** were characterized by (a) nucleoside composition after enzymatic digestion with snake venom phosphodiesterase and alkaline phosphatase, and (b) mass spectrometry.

**2:** (a) dG; (b)  $m/z = 694.8$  (MALDI-TOF, negative mode), calcd. for  $\text{C}_{29}\text{H}_{42}\text{N}_7\text{O}_9\text{PS}$ : 695.69.

**3:** (a) dG; (b)  $m/z = 701.2$  (MALDI-TOF, negative mode), calcd. for  $\text{C}_{30}\text{H}_{40}\text{N}_9\text{O}_9\text{P}$ : 701.64.

**4:** (a) dG; (b)  $m/z = 831.1$  (electrospray, negative mode), calcd. for  $\text{C}_{35}\text{H}_{49}\text{N}_{10}\text{O}_{10}\text{PS}$ : 832.83.

**Phac-His(Dnp)-Gly-Met-OH:** Solid-phase assembly using standard Boc methodology on Boc-Met-*O*-PAM-resin (PAM = phenylacetamidomethyl), followed by phenylacetylation of the *N*-terminus and cleavage of the peptide–resin bond by reaction with anhydrous HF (in the presence of 10% anisole) afforded the target tripeptide. No purification was required. Electrospray-MS (positive mode):  $m/z = 628.0$  [ $\text{M} + \text{H}$ ]<sup>+</sup>; calcd. for  $\text{C}_{27}\text{H}_{29}\text{N}_7\text{O}_9\text{S}$ : 627.59.

**Phac-His-Gly-Met-linker-<sup>5</sup>dCATGGCT (5):** The oligonucleotide was assembled on DMT-T-succinyl-sarcosyl-Ala-resin using the phosphite triester approach. The linker and tripeptide Phac-His(Dnp)Gly-Met-OH were subsequently incorporated as described above. Final deprotection was achieved by treatment with conc. aq.  $\text{NH}_3$ /dioxane (1:1; overnight, room temp.). **5** was purified by MPLC (gradient from 0 to 30% of B, the same solvent system as used for hybrids **2–4**). Nucleoside composition after enzymatic digestion: dC 2.09, dG 1.98, dT 2.04, dA 0.88. MALDI-TOF MS:  $m/z = 2717.6$  (negative mode); calcd. for  $\text{C}_{95}\text{H}_{126}\text{N}_{31}\text{O}_{48}\text{P}_7\text{S}$ : 2718.91.

**Ac-Lys-Met-Tyr(p<sup>3</sup>dACTAGT)-Pro-OH (6):** Nucleopeptide **6** was assembled according to the general procedure described in ref.<sup>[6]</sup> with minor modifications to prevent diketopiperazine formation (see text). Peptide–resin: Ac-Lys(Tfa)-Met(*O*)-Tyr-Pro-*O*-HMFS-Leu-resin. After oligonucleotide elongation using the phosphite triester methodology and removal of the 5'-DMT group, treatment of the nucleopeptide–resin with conc. aq.  $\text{NH}_3$ /dioxane (1:1) overnight at 55 °C afforded Ac-Lys-Met(*O*)-Tyr(p<sup>3</sup>dACTAGT)-Pro-OH. The target nucleopeptide, **6**, was then obtained by reduction of the sulfoxide (73 OD of hybrid, 1.0 mM in  $\text{H}_2\text{O}$ ; Ar atmosphere) with 20 equiv. of *N*-methylmercaptoacetamide for 96 h at 37 °C followed by MPLC purification (gradient from 0 to 30% of B, the same solvent system as used above). Nucleoside composition after enzymatic digestion: dC 1.14, dG 0.97, dT 2.09, dA 1.80; amino acid analysis after acid hydrolysis (6 M HCl in the presence of phenol and dithiothreitol, 1 h, 160 °C): Lys 1.05, Met 0.93, Tyr 0.97, Pro 1.05. MALDI-TOF MS (reflectron):  $m/z = 2430.4$ ; electrospray-MS:  $m/z = 607.1$  [ $\text{M} - 4\text{H}$ ]<sup>4+</sup>, 809.8 [ $\text{M} - 3\text{H}$ ]<sup>3+</sup>, 1215.0 [ $\text{M} - 2\text{H}$ ]<sup>2+</sup>; found 2432.3; calcd. for  $\text{C}_{86}\text{H}_{115}\text{N}_{27}\text{O}_{34}\text{P}_6\text{S}$ : 2432.74.

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- [1] J. D. Kahl, M. M. Greenberg, *J. Org. Chem.* **1999**, *64*, 507–510.
- [2] I. Schwope, C. F. Bleczynski, C. Richert, *J. Org. Chem.* **1999**, *64*, 4749–4761.
- [3] L. De Napoli, A. Messere, D. Montesarchio, G. Piccialli, E. Benedetti, E. Bucci, F. Rossi, *Bioorg. Med. Chem.* **1999**, *7*, 395–400.
- [4] A. Sakakura, Y. Hayakawa, H. Harada, M. Hirose, R. Noyori, *Tetrahedron Lett.* **1999**, *40*, 4359–4362.
- [5] S. Flohr, V. Jungmann, H. Waldmann, *Chem. Eur. J.* **1999**, *5*, 669–681.
- [6] J. Robles, M. Beltrán, V. Marchán, Y. Pérez, I. Travesset, E. Pedroso, A. Grandas, *Tetrahedron* **1999**, *55*, 13251–13263.
- [7] C. N. Tetzlaff, I. Schwope, C. F. Bleczynski, J. A. Stenberg, C. Richert, *Tetrahedron Lett.* **1998**, *39*, 4215–4218.
- [8] D. L. McMinn, M. M. Greenberg, *J. Am. Chem. Soc.* **1998**, *120*, 3289–3294.
- [9] S. Peyrottes, B. Mestre, F. Burlina, M. J. Gait, *Tetrahedron* **1998**, *4*, 12513–12522.
- [10] D. Gottschling, H. Seliger, G. Tarrasón, J. Piulats, R. Eritja, *Bioconjugate Chem.* **1998**, *9*, 831–837.
- [11] J. G. Harrison, S. Balasubramanian, *Nucleic Acids Res.* **1998**, *26*, 3136–3145.
- [12] M. A. Farrow, F. Aboul-ela, D. Owen, A. Karpeisky, L. Beigelman, M. J. Gait, *Biochemistry* **1998**, *37*, 3096–3108.
- [13] C. Guibourdenche, D. Seebach, F. Natt, *Helv. Chim. Acta* **1997**, *80*, 1–13.
- [14] E. Vivés, B. Lebleu, *Tetrahedron Lett.* **1997**, *38*, 1183–1186.
- [15] R. K. Bruick, P. E. Dawson, S. B. H. Kent, N. Usman, G. F. Joyce, *Chem. Biol.* **1996**, *3*, 49–56.
- [16] C.-H. Tung, K. J. Breslauer, S. Stein, *Bioconjugate Chem.* **1996**, *7*, 529–531.
- [17] M. Beltrán, G. B. Onoa, E. Pedroso, V. Moreno, A. Grandas, *J. Biol. Inorg. Chem.* **1999**, *4*, 701–707.
- [18] M. Beltrán, E. Pedroso, A. Grandas, *Tetrahedron Lett.* **1998**, *39*, 4115–4118.
- [19] Abbreviations: Aa = amino acid, Boc = *tert*-butoxycarbonyl, Bz = benzoyl, CNE = 2-cyanoethyl, DCC = *N,N'*-dicyclohexylcarbodiimide, DIEA = *N*-ethyl-*N,N*-diisopropylamine, DMAP = 4-dimethylaminopyridine, Dmf = dimethylaminomethylene, DMF = *N,N*-dimethylformamide, DMT = 4,4'-dimethoxytrityl, Dnp = 2,4-dinitrophenyl, Fmoc = 9-fluorenylmethoxycarbonyl, HATU = *N*-[(dimethylamine)-1*H*-1,2,3-triazole[4,5-*b*]pyridine-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate, HBTU = 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, HMFS = 2-(9-oxomethylfluorenyl)succinyl, HOBt = 1-hydroxybenzotriazole, Hse = homoserine, *i*Bu = isobutyl, MMT = 4-methoxytrityl, MPLC = medium-pressure liquid chromatography, MSNT = 1-(mesitylene-2-sulfonyl)-3-nitro-1*H*-1,2,4-triazole, NMI = *N*-methylimidazole, NMP = *N*-methylpyrrolidone, Nu = nucleoside, Phac = phenylacetyl, PS = polystyrene-*co*-1%-divinylbenzene resin functionalized as *p*-methylbenzhydrylamine, PyBOP = benzotriazole-1-yl-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate, R = resin (solid matrix), TBAF = tetrabutylammonium fluoride, TCA = trichloroacetic acid, Tfa = trifluoroacetyl, TFA = trifluoroacetic acid, TMG = tetramethylguanidine.
- [20] B. S. Sproat, M. J. Gait, in *Oligonucleotide Synthesis: A Practical Approach* (Ed.: M. J. Gait), IRL Press, Oxford, **1985**, pp. 104–106.
- [21] M. Beltrán, M. Maseda, J. Robles, E. Pedroso, A. Grandas, *Lett. Pept. Sci.* **1997**, *4*, 147–155.
- [22] T. Brown, C. E. Pritchard, G. Turner, S. A. Salisbury, *J. Chem. Soc., Chem. Commun.* **1989**, 891–893.
- [23] C. J. Bagley, K. M. Otteson, B. L. May, S. N. McCurdy, L. Pierce, F. J. Ballard, J. C. Wallace, *Int. J. Peptide Prot. Res.* **1990**, *36*, 356–361.
- [24] A. Thaler, D. Seebach, F. Cardinaux, *Helv. Chim. Acta* **1991**, *74*, 628–643.
- [25] P. Lloyd-Williams, F. Albericio, E. Giralt, in *Chemical Approaches to the Synthesis of Peptides and Proteins*, CRC Press, Boca Raton, **1997**, pp. 65–67.
- [26] D. S. Kemp, in *The Peptides: Analysis, Synthesis, and Biology*, vol. 1 (Major Methods of Peptide Bond Formation) (Eds.: E. Gross, J. Meienhofer), Academic Press, New York, **1979**, pp. 331, 339–340.
- [27] N. L. Benoiton, in *The Peptides: Analysis, Synthesis, and Biology*, vol. 5 (Special Methods in Peptide Synthesis, Part B) (Eds.: E. Gross, J. Meienhofer), Academic Press, New York, **1980**, pp. 169–175.
- [28] N. N. Polushin, B.-C. Chen, L. W. Anderson, J. S. Cohen, *J. Org. Chem.* **1993**, *58*, 4606–4613.
- [29] L. A. Carpino, A. El-Faham, C. Minor, F. Albericio, *J. Chem. Soc., Chem. Commun.* **1994**, 201–203.
- [30] G. Barany, R. B. Merrifield, in *The Peptides: Analysis, Synthesis, Biology*, vol. 2 (Special Methods in Peptide Synthesis, Part A) (Eds.: E. Gross, J. Meienhofer), Academic Press, New York, **1980**, pp. 223–229.
- [31] H. Lamthanh, C. Roumestand, C. Deprun, A. Ménez, *Int. J. Peptide Protein Res.* **1993**, *41*, 85–95.
- [32] E. H. Amonoo-Neizer, S. K. Ray, R. A. Shaw, B. C. Smith, *J. Chem. Soc.* **1965**, 4296–4300.
- [33] D. M. Andrews, J. Kitchin, P. W. Seale, *Int. J. Peptide Prot. Res.* **1991**, *38*, 469–475.
- [34] *Novabiochem Catalog & Peptide Synthesis Handbook*, Technical Notes, **1999**.
- [35] R. A. Houghten, C. H. Li, *Anal. Biochem.* **1979**, *98*, 36–46.
- [36] S. Futaki, T. Taike, T. Yagami, T. Akita, K. Kitagawa, *Tetrahedron Lett.* **1989**, *30*, 4411–4412.
- [37] W. Beck, G. Jung, *Lett. Pept. Sci.* **1994**, *1*, 31–37.
- [38] M. W. Pennington, M. Byrnes, *Peptide Res.* **1995**, *8*, 39–43.
- [39] E. Nicolás, M. Vilaseca, E. Giralt, *Tetrahedron* **1995**, *51*, 5701–5710.
- [40] J. Robles, M. Maseda, M. Beltrán, M. Concernau, E. Pedroso, A. Grandas, *Bioconjugate Chem.* **1997**, *8*, 785–788.
- [41] E. Kuyl-Yeheskiely, C. M. Tromp, A. W. M. Lefeber, G. A. van der Marel, J. H. van Boom, *Tetrahedron* **1988**, *44*, 6515–6523.
- [42] F. Rabanal, E. Giralt, F. Albericio, *Tetrahedron Lett.* **1992**, *39*, 1775–1778.
- [43] M. Gairi, P. Lloyd-Williams, F. Albericio, E. Giralt, *Tetrahedron Lett.* **1990**, *31*, 7363–7366.

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