148. The CAMET and CASET Links for the Synthesis of Protected Oligopeptides and Oligodeoxynucleotides on Solid and Soluble Supports[1] [2]

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Summary

Simple bifunctional carboxy- and phospho-protecting groups are described which allow the attachment of protected amino acids and nucleotides to soluble or insoluble carriers. After chemical synthesis with conventional procedures, the completed oligopeptides or oligonucleotides can be detached by base-catalyzed β-elimination, leaving other protecting groups intact. These protected biopolymer segments can then be purified, characterized, and used for further synthetic work by virtue of their free carboxy or phospho groups. It is also possible to deprotect peptides and nucleotides on the supports: this procedure may be used for the preparation of affinity-chromatographic materials.

Since its first announcement by *Merrifield* in 1962 [1b], the 'solid-phase' method has become one of the most popular techniques for the synthesis of polypeptides on a laboratory scale. The polymer-support principle is also in use for the preparation of polynucleotides [2].

Many of the still remaining difficulties and limitations could be eliminated if the method would allow the facile preparation of protected oligomeric fragments with free terminal carboxy or phospho groups. These could be purified, duly characterized, and then used as intermediates for the preparation of longer chains either by classical condensation in solution [2] [3] or on solid supports [4].

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[1] Reported at the 4th *Max-Bergmann* Konferenz, October 10, 1983, Bolzano, Italy. Part of the doctoral thesis of *E.F.*

[2] Abbreviations for amino acids, peptides, and nucleotides are according to the *IUPAC-IUB* recommendations [1a]. Chp is 4-chlorophenyl, Mmt (MeOTr) is 4-methoxytrityl, Tcp is 2,4,5-trichlorophenyl, and Pfp is pentafluorophenyl; DCC denotes *N,N'*-dicyclohexylcarbodiimide. H-CAMET-OH, H-CASET(1)-OH, and H-CASET(2)-OH are 2-(4-carboxyphenylmercapto)ethanol (IUPAC name: 4-(2-hydroxyethylthio)benzoic acid), 2-(4-carboxyphenylsulfinyl)ethanol (IUPAC name: 4-(2-hydroxyethylsulfinyl)benzoic acid), and 2-(4-carboxyphenylsulfonyl)ethanol (IUPAC name: 4-(2-hydroxyethylsulfonyl)benzoic acid), respectively. Substituents on the alcoholic O-atom of the links appear to the left, those on the carboxy group to the right of the symbols CAMET, CASET(1), and CASET(2), thus following in principle the rules for amino acids and peptides.

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To this end, spacer molecules or 'links' must be placed between the growing biopolymer and the supporting resin. Such links should have the following properties: *a*) they should be able to form derivatives of amino-acid carboxy and nucleotide phospho groups which can easily be attached to functional groups of the support, *b*) they must be resistant against the reagents and solvents employed during chain elongation, and *c*) they must be selectively cleaved to release the intact, protected oligomeric biopolymer segment with a free carboxy or phospho group.

Many links have been reported [5], but few have the required properties. A recent and very sophisticated approach to the problem of polypeptides is that of *Tam et al.* [6]. For the solidphase synthesis of oligodeoxynucleotides, the succinyl group [7] is widely used for linking 3'-OH group of the first nucleotide to the amino function of derivatized solid supports. However, this link cannot be used to prepare 3'-phosphates and is, furthermore, rather unstable during chain elongation [8].

Since a few years, we have been using very simple and versatile links for the synthesis of protected oligopeptides and oligonucleotides on resin supports (solid phase) or on chromophoric carriers (solution synthesis). They are: 2-(4-carboxyphenylmercapto)ethanol (H-CAMET-OH), 2-(4-carboxyphenylsulfinyl)ethanol (H-CASET(1)-OH), and 2-(4-carboxyphenylsulfonyl)ethanol (H-CASET(2)-OH)[2] *(Figure,* see also *Scheme 1)*.

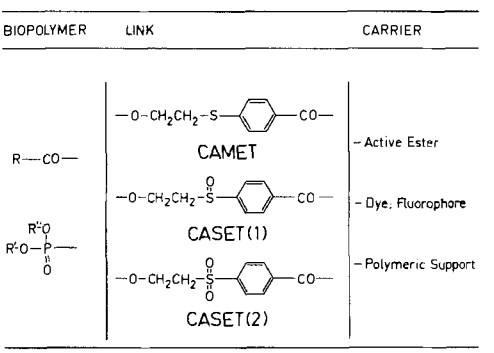


Figure. *The bifunctional links, CAMET, CASET(1), and CASET(2), for connecting biopolymers to soluble or insoluble carrier units.* RCO– is an amino-acid or peptide residue. R' is a nucleoside or (oligo-)nucleotide. R'' is H (in the 'diester' synthetic approach) or a protecting group such as 4-chlorophenyl (in the 'triester' mode).

The carboxy groups can be activated by esterification with 2,4,5-trichlorophenol, pentafluorophenol, *N*-hydroxysuccinimide, and the like (see *Scheme 2*). The alcoholic OH groups can be esterified with carboxy or phosphate groups of protected amino acids or nucleotides by conventional methods (see *Schemes 3, 4,* and *7*). The resulting amino-acid or nucleotide derivatives can then be reacted with amino groups of functionalized resins or other supports, or with amines of low molecular weight (shown for nucleotides in *Schemes 5* and *6*).

After elimination of the acid-labile O^5'- or N^a-protecting groups and chain elongation, the protected or deprotected biopolymer can be cleaved from the CASET(1) and CASET(2) links by base-catalyzed β-elimination (*e.g. Schemes 5* and *6; syntheses of*

longer oligonucleotides and peptides using a variety of strategies shall be reported separately). One particular advantage of the CAMET/CASET family is the easy accessibility of different oxidation stages: each degree of oxidation entails a different degree of stability against base-catalyzed β-elimination.

Nucleotide derivatives of CAMET are very stable towards acid and base so that leaking of intermediates during the synthesis is no problem. The stability is such that oligonucleotide triesters can readily be converted to diesters with base and 4-nitrobenzaldehyde oxime [9]. This possibility allows the application of *Pfleiderer*'s strategy of synthesis in solution [10] to nucleotides on a solid support, in our case removal of Chp and chain elongation at the phosphate group bound to the CAMET link.

After completion of nucleotide synthesis on a CAMET support, the link can be oxidized to the sulfoxide stage, CASET(1), with $NaIO_4$ [11] without harming the attached deoxynucleotides. *N*-Chlorosuccinimide also oxidizes to the sulfoxides, and 3-chloroperbenzoic acid produces the sulfones; however, the latter oxidant may also react with nucleotides and is not generally recommended. The *p*-carbamoyl substituent enhances the base-catalyzed β-elimination reaction of CASET(1) derivatives considerably, so that an oxidation to CASET(2) is unnecessary. Thus, phosphotriesters linked by CASET(1) are stable against Et_3N in pyridine solution, but are cleaved by tetramethylguanidine, whereas phosphodiesters require the action of MeONa. Hence, nucleotide synthesis by the triester or diester methods may also be accomplished using the CASET(1) link instead of CAMET, thus eliminating the oxidation step.

Peptides are poorer leaving groups than phosphodi- or -triesters, and CASET(2) is the link of choice for peptide synthesis. The cleavage of protected peptides from CASET(2) supports is effected by 0.1N NaOH or $Ba(OH)_2$ in a matter of minutes, or somewhat slower by tetramethylguanidine in pyridine (detailed report in preparation). CAMET is less useful for peptide synthesis, because its oxidation to the sulfoxide or sulfone stages will also oxidize many peptides. However, for the preparation of resins coated with synthetic peptides (for affinity chromatography), the CAMET link is very useful (to be reported elsewhere).

Tesser [12] was the first to devise a link between the *Merrifield*-type polystyrene resin and the growing polypeptide chain that allowed a facile detachment of the biopolymer by base-catalyzed β-elimination. He reacted β-mercaptoethanol with chloromethylated polystyrene resin to produce the thioether, $RCH_2SCH_2CH_2OH$ (R–CH_2-stands for the resin-containing methylene substituents on phenyl groups). A protected amino acid was then esterified with the OH groups of the substituted resin and the product used as the starting point for solid-phase peptide synthesis. The completed peptide was detached by oxidation of the sulfur to the sulfone stage, followed by base-catalyzed β-elimination.

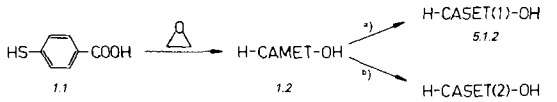
The very same type of 2-hydroxyethylthiomethylene resin was recently prepared in Moscow [13], oxidized to the sulfone stage, and used for the solid-phase synthesis of oligonucleotide 3'-phosphates by the triester method. Cleavage by β-elimination yielded protected tetranucleotides with 3'-(4-chlorophenylphosphate) groups. The Chp protecting group can not be removed therefrom, but the products can be used as building blocks for further triester syntheses.

The main disadvantage of this type of approach is common to all procedures in which the link is attached to the resin before the amino acid or nucleotide is introduced. They lead to heterogeneous resins with respect to functionality, *e.g.* RCH_2Cl, RCH_2OH, $RCH_2SCH_2CH_2OH$, and thus to biopolymer chains with different points of attachment (see the discussion of the general problem *e.g.* by *Birr* [5]). The problem with oxidation during peptide synthesis has been pointed out above; the problem of nucleotide leakage from the sulfonyl resin has not been addressed explicitly by the Russian authors, but is apparently less serious than from CASET(2) resins, because of a lesser reactivity of nucleotidyl-$OCH_2CH_2SO_2CH_2C_6H_5$ with base (described in the doctoral thesis of *E.F.* at the ETHZ, in preparation).

'Safety catch' systems similar to that of *Tesser* [12] and with similar limitations have been devised for nucleotide synthesis on polymeric carriers. *Brandstetter et al.* [14] functionalize polyethylene glycol with 2-(4-aminophenylthio)ethanol by connection through the amino group. A protected deoxynucleoside 5'-phosphate is attached through the phosphate. Oxidation to the sulfone with *N*-chlorosuccinimide and β-elimination releases the deoxyoligonucleotide 5'-phosphate. *Gait & Sheppard* [15] use a solid carrier functionalized by reaction with the pentachlorophenyl ester of 4-(2-hydroxyethylthio)dihydrocinnamic acid and proceed according to [14]. It is quite difficult to achieve clearcut oxidations to the sulfone stage with *N*-chlorosuccinimide, and the sulfoxide stages of the two links are perhaps not very suitable for β-elimination because of a lack of electronic activation by their *p*-substituents.

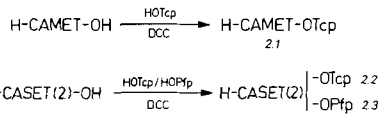
Results. – 1) *The Educts H-CAMET-OH and H-CASET(2)-OH (*see *Scheme 1*[4]*)).* 4-Mercaptobenzoic acid [16–19] was condensed with ethylene oxide to give H-CAMET-OH, which was oxidized with H_2O_2 in the presence of sodium tungstate to H-CASET(2)-OH *(b)*. Both products had been prepared earlier using a somewhat different procedure [20]. H-CASET(2)-OH was also prepared using 3-chloroperbenzoic acid as oxidant. H-CASET(1)-OH was produced from H-CAMET-OH by oxidation with $NaIO_4$ *(a)*, but was not isolated; its presence was implied from its behavior on TLC.

Scheme 1. *Preparation of Starting Materials*



[a]) $NaIO_4$. [b]) H_2O_2/Na_2WO_4

Scheme 2. *Preparation of CAMET- and CASET(2)-Active Esters*[4])



2) *Active Esters of CAMET and CASET(2) (*see *Scheme 2)*. These are key intermediates for much of the work reported here. They were obtained as crystalline compounds in quite good yields (60–80%) with DCC in dioxane or *N,N*-dimethylformamide (see *Exper. Part*[4])).

3) *Nucleotide Derivatives of CAMET and CASET(2) Active Esters (*see *Scheme 3* and *4)*. Stepwise condensation of 4-chlorophenyl phosphorodichloridate [21] with 1,2,4-triazole, 5'-*O*-(4-methoxytrityl)thymidine and H-CAMET-OTcp *(Exper. 3.1)* gave a good yield of the nucleotide triester Mmt-dTp(Chp)-CAMET-OTcp. A similarly good result was obtained in the preparation of the corresponding diester Mmt-dTp-CAMET-OTcp according to *Scheme 4 (Exper. 3.2)*. A condensation similar to that of *Scheme 3*, but using H-CASET(2)-OTcp as active ester component *(Exper. 3.3)* gave Mmt-dTp(Chp)-CASET(2)-OTcp in variable yields, which was mainly due to the β-elimination of the nucleotide caused by the basic solvent, pyridine. These experiments showed that the CASET(2) link was quite useless for our purposes in nucleotide synthesis (but not for peptide synthesis, see below).

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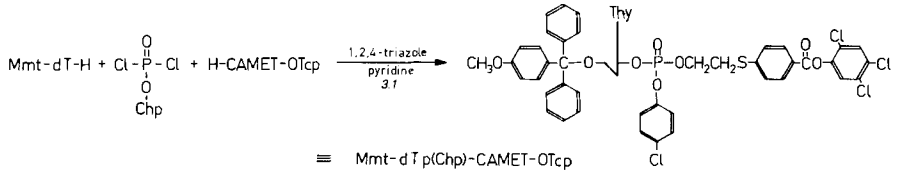
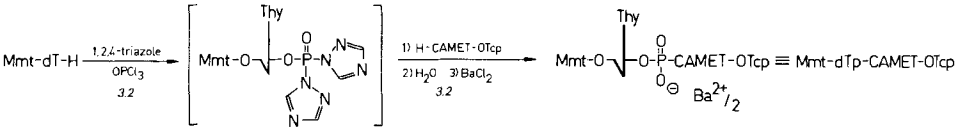
[4]) Numbers in italics in the *Schemes* refer to the *Exper. Part.*

Scheme 3. *Preparation of a Nucleotide Triester Derivative of a CAMET-Active Ester*[4])

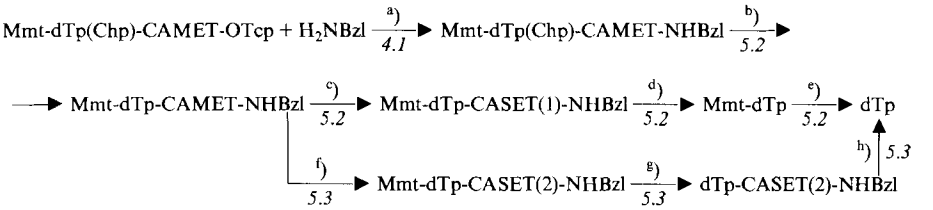


Scheme 4. *Preparation of a Nucleotide Diester Derivative of a CAMET-Active Ester*[4])



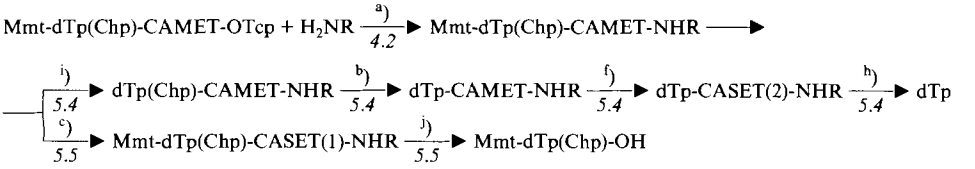
4) *Condensation of Nucleotidyl-CAMET Active Esters with Carrier Amines* (see *Schemes 5* and *6, Step a)*. This type of reaction was exemplified by the condensation of Mmt-dTp(Chp)-OTcp with benzylamine *(Exper. 4.1)* and with an *Atherton-Sheppard*-type polydimethylacrylamide resin functionalized with ethylenediamine [22] *(Exper. 4.2)*. The triester benzylamide, Mmt-dTp(Chp)-CAMET-NHCH$_2$C$_6$H$_5$ was purified and analyzed in a conventional manner (yield 76%). The amino groups of the resin reacted quantitatively with the active ester in pyridine as demonstrated by a quantitative spectrophotometric assay for the 4-methoxytrityl residue.

Scheme 5. *Condensation of a Nucleotide Triester Derivative of a CAMET-Active Ester with a Soluble Carrier* (H_2NBzl is benzylamine), *Conversion of the Product to the Diester, Oxidations, and β-Eliminations of a Protected and an Unprotected Nucleoside 3'-Phosphate*[4])



[a]) Condensation in pyridine. [b]) 4-Nitrobenzaldehyde oxime/tetramethylguanidine in aq. dioxane. [c]) $NaIO_4$ in aq. dioxane. [d]) 0.1N MeONa in pyridine. [e]) [g]) AcOH. [f]) 3-Chloroperbenzoic acid in dioxane. [h]) 0.1N NaOH.

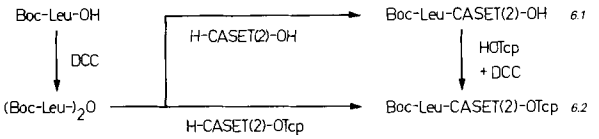
Scheme 6. *Condensation of a Nucleotide Triester Derivative of a CAMET-Active Ester with an Insoluble Carrier* (H_2NR is a polydimethylacrylamide resin functionalized with ethylenediamine [22]) *and Processing to Release dTp or Mmt-dTp(Chp)-OH*[4])



[a]) [b]) [c]) [f]) [h]) See *Scheme 5*. [i]) TsOH in $CHCl_3$/MeOH 7:3. [j]) Tetramethylguanidine in pyridine.

5) *Oxidation of Nucleotidyl-CAMET-amides to the Sulfoxide and Sulfone Stages and β-Elimination of Nucleotides* (see *Schemes 5* and *6*). The oxidation of H-CAMET-OH was investigated in preliminary experiments (*Exper. 5.1*). As judged by TLC 3-chloroperbenzoic acid in dioxane oxidized it quantitatively to pure H-CASET(2)-OH. Pure H-CASET(1)-OH was apparently produced in quantitative yield either with *N*-chlorosuccinimide in dioxane/phosphate buffer at pH 7.5 or with $NaIO_4$ in dioxane/H_2O as indicated by TLC. Both the oxidation to the sulfoxide stage (*Step c*) and to the sulfone stage (*Step f*) were applied in order to demonstrate the β-elimination of protected nucleotides (*Steps d, h,* and *j*).

Scheme 7. *Preparation of Amino-Acid Derivatives of CASET(2) and its Active Esters*[4])



6) *Amino-Acid Derivatives of CASET(2) and Active Esters, Scheme 7.* – *N-t*-Butoxycarbonylleucine (Boc-Leu-OH) was converted to its symmetrical anhydride with DCC, and this was reacted with H-CASET(2)-OTcp in AcOEt and pyridine to give Boc-Leu-CASET(2)-OTcp in good yield. This product was identical with that prepared from Boc-Leu-CASET(2)-OH (free acid) and 2,4,5-trichlorophenol with DCC. The latter reaction type was also carried out with Boc-Gly-CASET(2)-OH and Boc-D-Ala-CASET(2)-OH. Boc-Phe-CASET(2)-OH, also prepared from the symmetrical Boc-amino-acid anhydride, was converted to its *N*-hydroxysuccinimide derivative in order to demonstrate the possibility of preparing other active esters.

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Experimental Part

General. M.p. were determined in open capillaries and are uncorrected. Evaporation means removal of solvents from dissolved products in a rotary evaporator at reduced pressure (0.01–10 Torr) and moderate temp.; coevaporation is the same after addition of another solvent to produce an azeotropic mixture. Solvent ratios are in volume parts. TLC, on plates coated with silica gel (sil) or cellulose (cel) was performed normally or after running the plate containing the material to be analyzed once with Et_2O to improve separations (eth). R_f values were determined in the following solvent systems: AW (acetone/H_2O 8:2); BAW 523 and BAW 1013 (BuOH/AcOH/H_2O, 5:2:3 and 10:1:3, respectively); CE ($CHCl_3$/AcOEt, 1:1); CIA ($CHCl_3$/i-PrOH/AcOH, 80:15:15); CM ($CHCl_3$/MeOH, 95:5); CMA ($CHCl_3$/MeOH/AcOH 95:5:3); CMPW ($CHCl_3$/MeOH/pyridine/H_2O, 32:8:4:1); CMT ($CHCl_3$/MeOH/Et_3N, 90:5:5); EPW (AcOEt/pyridine/H_2O 20:20:11); INW (i-PrOH/conc. NH_3/H_2O; 7:1:2). Well-known educts, intermediates, and procedures of peptide chemistry are described in *Houben-Weyl* [13] where experimental details are given and the literature is summarized.

1. The Educts CAMET and CASET(2). – 1.1. *4-Mercaptobenzoic Acid.* Preparation from 4-aminobenzoic acid *via* the diazonium salt and the ethyl xanthate essentially as described by *Cummings* [16] and taking precautions against oxidation according to *Gialdi et al.* [17], *Campaigne & Meyer* [18], and *Grice & Owen* [19].

1.2. *2-(4-Carboxyphenylmercapto)ethanol (CAMET)*[2]. A solution of 4-mercaptobenzoic acid (4.6 g, 30 mmol) in dry dioxane (40 ml) was cooled to 0° and treated first with ethylene oxide (12.5 g, 284 mmol) and then with Et_3N (4.2 ml, 30 mmol). The mixture was kept in a pressure flask at r.t. for 40 h during which a brown, oily precipitate was formed. The solvents were evaporated, the residue dissolved in a small amount of ice-cold H_2O and acidified to pH 2 with 1N HCl. The solid product was gathered by filtration, dried, and recrystallized from i-PrOH/$(i\text{-Pr})_2O$ (1:1, 40 ml) by careful addition of pentane and from i-PrOH/H_2O after treatment with a trace of charcoal. Colorless needles of CAMET (3.5 g, 58.8%). M.p. 143–144° ([20]: 151–153°, prepared from 2-chloroethanol). Anal. calc. for $C_9H_{10}O_5S$ (198.24): C 54.54, H 5.09, S 16.18; found: C 54.44, H 5.01, S 15.98.

1.3. *2-(4-Carboxyphenylsulfonyl)ethanol (CASET(2))*[2]. A solution of CAMET (5 g, 25 mmol) and $Na_2WO_4 \cdot 2H_2O$ (150 mg) in dioxane/H_2O 1:1 (100 ml) was heated to 60° and treated dropwise with a 30% aq. solution (30 g) of H_2O_2 (250 mmol). After a total reaction time of 24 h at 60°, the dioxane was removed by evaporation until crystallization had begun. The crystals were gathered after cooling for a few h at 0° and recrystallized from H_2O after treatment with charcoal. Colorless needles of CASET(2) (3.8 g, 65.5%). M.p. 191° ([20]: 193–194°, oxidation with sodium hypochlorite). Anal. calc. for $C_9H_{10}O_5S$ (230.24): C 46.96, H 4.38, S 13.93; found: C 46.90, H 4.39, S 13.64.

2. Active Esters of CAMET and CASET(2). – 2.1. *CAMET-OTcp (2-[4-(2,4,5-trichlorophenoxycarbonyl)phenylmercapto]ethanol = 2,4,5-Trichlorophenyl 4-(2-Hydroxyethylthio)benzoate).* CAMET (2.973 g, 15 mmol) and 2,4,5-trichlorophenol (3.554 g, 18 mmol) were dissolved in dioxane (60 ml) and treated dropwise with a solution of DCC (3.714 g, 18 mmol) in dioxane (12 ml). After 15 h, the precipitate of dicyclohexylurea was removed by filtration, washed generously with dioxane, and the combined filtrate evaporated to a thick syrup. The syrup was dissolved in a small amount of $CHCl_3$ (*ca.* 15 ml) and the solution added dropwise, with stirring, to ice-cold hexane (200 ml). The voluminous precipitate was gathered by filtration and recrystallized from $CHCl_3$/cyclohexane. For analysis, the product was chromatographed through a column of silica gel (85 g) with $CHCl_3$: 3.45 g (61%); m.p. 110°; TLC (sil): R_f 0.7 (CM). UV (MeCN): 303 (4.40). Anal. calc. for $C_{15}H_{11}Cl_3OS$ (377.68): C 47.70, H 2.94, Cl 28.21; found: C 47.61, H 2.82, Cl 28.36.

2.2. *CASET(2)-OTcp (2-[4-(2,4,5-trichlorophenoxycarbonyl)phenylsulfonyl]ethanol = 2,4,5-Trichlorophenyl 4-(2-Hydroxyethylsulfonyl)benzoate).* CASET(2) (1.151 g, 5 mmol) and 2,4,5-trichlorophenol (1.85 g, 6 mmol) were dissolved in DMF (30 ml) and treated dropwise with a solution of DCC (1.238 g, 6 mmol) in the same solvent (20 ml). After 15 h, the precipitate of dicyclohexylurea was removed by filtration, washed with fresh solvent, and the filtrate evaporated to a thick syrup. The residue was crystallized twice from EtOH: 1.3 g (63%); m.p. 127° TLC (sil): $R_f = 0.52$ (CMA), 0.29 (CE). Anal. calc. for $C_{15}H_{11}O_5SCl_3$ (409.67): C 43.98, H 2.71, Cl 25.96; found: C 43.96, H 2.80, Cl 25.64.

2.3. *CASET(2)-OPfp (2-[4-(pentafluorophenoxycarbonyl)phenylsulfonyl]ethanol = Pentafluorophenyl 4-(2-Hydroxyethylsulfonyl)benzoate).* CASET(2) (2.302 g, 10 mmol), pentafluorophenol (2.025 g, 11 mmol), and DCC (2.476 g, 12 mmol) were reacted in dioxane (100 ml) as described above for CAMET-OTcp. Colorless crystals from i-PrOH, yield 3.1 g (78%); m.p. 120–121°. TLC (sil): R_f 0.48 (CMA), R_f 0.26 (CE). Anal. calc. for $C_{15}H_9F_5O_5S$ (396.29): C 45.46, H. 2.29, S 8.09; found: C 45.53, H 2.25, S 8.21.

3. Nucleotide Derivatives of CAMET and CASET(2) Active Esters. – 3.1. *Mmt-dTp(Chp)-CAMET·OTcp (5'-O-(4-Methoxytrityl)thymidine 3'-{4-chlorophenyl 2-[4-(2,4,5-Trichlorophenoxycarbonyl)phenylthio]ethyl Phosphate}).* 1,2,4-Triazole (180 mg, 2,6 mmol) in pyridine (1.5 ml) was treated with 4-chlorophenyl phosphorodichloridate (319 mg, 1.3 mmol) and cooled in ice. Then, a solution of 5'-*O*-(4-methoxytrityl)thymidine (514 mg, 1 mmol) in pyridine (1.5 ml) was added dropwise to the cold solution of the phosphorylation reagent. The flask was rinsed with a second portion of pyridine (1.5 ml). After 45 min in the ice bath, the solution was complemented with CAMET-OTcp (755 mg, 2 mmol). After another 4 h, the solvent was evaporated and the evaporation repeated after adding a similar volume of toluene. The residue was then dissolved in $CHCl_3$ (100 ml) and washed with phosphate buffer (0.1M, pH 7). The org. phase was dried and evaporated twice, the second time after adding toluene. The residue was again dissolved in $CHCl_3$ and chromatographed through a silica gel column (20 g, 2 cm diameter) with $CHCl_3$: 911 mg (85%) of a colorless solid. TLC (sil): R_f 0.75 (CM). UV (MeCN): 297 (4.301). ^{1}H-NMR confirmed identity and purity. Anal. calc. for $C_{51}H_{43}N_2Cl_4O_{11}PS$ (1064.5): C 57.49, H 4.04, N 2.63; found: C 56.96, H 4.15, N 2.96.

3.2. *Mmt-dTp-CAMET-OTcp (5'-O-(4-Methoxytrityl)thymidine 3'-{2-[4-(2,4,5-Trichlorophenoxycarbonyl)-phenylthio]ethyl Phosphate}).* 1,2,4-Triazole (165.6 mg, 2.4 mmol) in a 10 ml round-bottomed flask was dissolved in *ca.* 4 ml of pyridine, and the solvent evaporated to remove H_2O. The residue was then dissolved in fresh, dry pyridine (1.1 ml) and treated with phosphoryl chloride (78 mg, 0.508 mmol). Immediately after the

addition from a thin pipette, the flask was stoppered and placed in ice. To this cold solution, 5'-*O*-(4-methoxytrityl)thymidine (205,6 mg, 0.4 mmol), dissolved in pyridine (1 ml), was added dropwise. The mixture was then kept at 20° for 45 min. At this time, no more Mmt-dT could be detected by TLC (sil, CMT), and the product remained at the starting point. A solution of CAMET-OTcp (232.5 mg, 0.616 mmol; freshsly dried by evaporation of a pyridine solution) in pyridine (1.5 ml) was dropped into the phosphorylation mixture. After 1.5 h at 20°, a new main product spot was observed by TLC (sil, R_f 0.35 (CMT)). The whole mixture was then slowly dropped into vigorously stirred H_2O (25 ml) and kept for 30 min at 20°. A slight turbidity was dissolved to the stage of slight opalescence by adding pyridine. This solution was then dropped into an ice-cold solution of $BaCl_2$ (1 g) in H_2O (100 ml). The very fine precipitate was coagulated by warming the solution to 30° and cooling it to 3°. After stirring for 10 min at 3°, the precipitate was gathered in a sintered glass funnel *(G2)* and washed with a small amount of ice-cold H_2O. The barium salt was suspended in pyridine, and the pyridine evaporated. Traces of pyridine were removed by coevaporation with toluene to give a hard, frothy residue (350 mg, *ca.* 84% yield). TLC revealed that this crude product contained mostly the phosphodiester (R_f 0.38) along with trace amounts of Mmt-Tp (R_f 0), Mmt-OH (R_f 0.95), and another, unidentified compound (R_f 0.52), but no Mmt-Tp-CAMET-OH (R_f 0.30) produced by hydrolysis of the active ester moiety (TLC, sil, CMT). Purification of the material was achieved by chromatography (silica gel, CMPW) through short columns with relatively large diameter. CMPW has the advantages of not hydrolyzing the active ester (no hydrolysis observed in 10 h at 20°, about 10% in 20 h), good resolution, and large capacity. The product was readily hydrolyzed to Mmt-Tp-CAMET·OH with aqueous KOH (see above). It was pure by TLC (sil): R_f 0.35 (CMT), 0.40 (CMPW), and 0.90 (reversed phase silica gel, *Whatman KC 18 F;* AW). It was not further characterized, but successfully used in dinucleotide synthesis, *e.g.* for the preparation of the dinucleotide Mmt-dTp (CAMET-OTcp)-dT-OR (R=*t*-butyldimethylsilyl; to be published).

3.3. *Mmt-dTp(Chp)-CASET(2)-OTcp(5'-O-(4-Methoxytrityl)thymidine 3'-{4-Chlorophenyl 2-[4-(2,4,5-Trichlorophenoxycarbonyl)phenylsulfonyl]ethyl Phosphate}).* 1,2,4-Triazole (35.5 mg, 0.514 mmol) in pyridine (1 ml) was treated with 4-chlorophenyl phosphorodichloridate (77 mg, 0.314 mmol) and then cooled in ice. A solution of 5'-*O*-(4-methoxytrityl)thymidine (100 mg, 0.195 mmol) in pyridine (1 ml) was added dropwise and the flask rinsed once with pyridine (1 ml). After 40 min in the ice bath, the solution containing the phosphodiester triazolide was complemented with CASET(2)-OTcp (143 mg, 0.349 mmol). After 5 h at 20°, the pyridine was evaporated and then co-evaporated with toluene. The residue was dissolved in $CHCl_3$ (50 ml) and extracted with phosphate buffer (0.1M, pH 7). The dried org. phase was evaporated and the residue chromatographed through a column of silica gel (20 g; 2 cm diameter) with $CHCl_3$. After washing with $CHCl_3$ (200 ml), the product was eluted with $CHCl_3$/MeOH, 99:1. The yields of colorless solid were 15–80%, and decreased with time of contact with base (mainly pyridine) TLC (sil): R_f 0.7 (CM). UV (MeCN): 268 (4.05). ^{1}H-NMR spectra confirmed identity and purity. Anal. calc. for $C_{51}H_{43}Cl_4N_2O_{13}PS$ (1096.5): C 55.89, H 3.95, N 2.55; found: C 56.20, H 4.07, N 2.36.

4. Condensation of Nucleotidyl-CAMET Active Esters with Carrier Amines. – 4.1. *Mmt-dTp(Chp)-CAMET-NHBzl (5'-O-(4-Methoxytrityl)thymidine 3'-{4-chlorophenyl 2-[4-(Benzylaminocarbonyl)phenylthio]-ethyl Phosphate}).* Mmt-dTp(Chp)-CAMET-OTcp (250 mg, 0.235 mmol) was dissolved in pyridine (1 ml) and treated with benzylamine (0.6 ml, 5.5 mmol). After 2.5 h, no more educt was seen on TLC. The pyridine was removed by evaporation and repeated coevaporation with toluene. The residue was dissolved in $CHCl_3$ and washed with phosphate buffer (0.1M, pH 7). The org. phase was dried and evaporated. The residue was dissolved in $CHCl_3$ and chromatographed through a silica gel column (18 g, 2.5 cm diameter) with $CHCl_3$. The amorphous product was transformed into a powder by dripping its $CHCl_3$ solution into cold hexane. Yield 175 mg (76%). TLC (sil, eth), R_f 0.65 (CM). UV (MeCN): 275 (4.37). Anal. calc. for $C_{52}H_{49}ClN_3O_{10}PS$ (974): C 64.07, H 5.03, N 4.31; found: C 64.56, H 5.37, N 4.49.

4.2. *Condensation of Mmt-dTp(Chp)-CAMET-OTcp with a Polydimethylacrylamide Resin Functionalized with Ethylenediamine* [22] *(0.34 mmol·g^{-1} of free amino groups).* Benzotriazol-1-ol containing about 20% of H_2O (90 mg, *ca.* 0.6 mmol) was dried by coevaporation with pyridine. The residue was dissolved in dry pyridine (1 ml) and treated with a solution of Mmt-dTp(Chp)-CAMET-OTpc (380 mg, 0.357 mmol) in pyridine (9ml). The functionalized resin (300 mg, 0.107 mmol of free amino groups) was added and the suspension agitated for 15 h at 20°, after which the *Kaiser* test [23] for free amino groups was negative. The resin was washed repeatedly with pyridine and then suspended in the same solvent (10 ml). The amount of bound nucleotide was determined essentially according to *Schaller* [24] by spectrophotometric assay of the 4-methoxytrityl group. An aliquot of the pyridine suspension of the resin (170 µl) was filtered, and the resin washed repeatedly with Et_2O and dried *in vacuo*. This sample (7.37 mg) was treated with a mixture of EtOH/70% $HClO_4$ 4:6 for exactly 15 min, filtered,

and the UV/VIS compared with that of 4-methoxytrityl chloride in the same solvent mixture (λ_{max} at 474 nm). A substitution rate of 105.4% was found. To proceed according to usual experimental protocols for solid phase synthesis, 'unreacted' functional groups were masked by treating the resin with a great excess of Ac_2O (about 1 g) in pyridine (10 ml). After agitating the mixture for 2 h at 20°, the resin was removed by filtration, washed 10 times with pyridine (10 ml each), and stored under pyridine.

5. Oxidation of Nucleotidyl-CAMET-amides to the Sulfoxide and Sulfone Stages. β-Elimination of Nucleotides. – 5.1. *Preliminary Experiments with the Oxidation of H-CAMET-OH to H-CASET(2)-OH and H-CASET(1)-OH.* – 5.1.1. *CASET(2) with 3-Chloroperbenzoic Acid.* A solution of CAMET (32 mg, 0.16 mmol) in dioxane (2 ml) was frozen at 0°. A solution of 3-chloroperbenzoic acid (80 mg, 0.46 mmol) in dioxane (0.6 ml) was added, and the mixture was left to thaw and kept for 2 h at 20°. The product was pure as judged by TLC(sil) and was identical with an anal. sample of CASET(2) from *1.3;* colorless needles; R_f 0.5(CIA), 0.07(CMA), 0.52(EPW).

5.1.2. *CASET(1) with N-Chlorosuccinimide or Sodium Metaperiodate.* CAMET (20 mg, 0.1 mmol) was added either to a solution of *N*-chlorosuccinimide (106 mg, 0.8 mmol) in 2 ml of dioxane/0.06M phosphate buffer (pH 7.5) 1:1, or to a solution of $NaIO_4$ (214 mg, 1 mmol) in 4 ml of dioxane/H_2O 1:1. In both experiments, after 30 min at 20°, all CAMET had been converted to a single new product (TLC(sil CIA): R_f 0.45), distinctly different from CAMET (R_f 0.75) and CASET(2) (R_f 0.50). The product was assumed to be the sulfoxide CASET(1), but not further characterized.

5.2. *Partial Deprotection of the Triester, Mmt-dTp(Chp)-CAMET-NHBzl to the Diester, Mmt-dTp-CAMET-NHBzl, Periodate Oxidation, β-Elimination and Acidolysis to dTp.* A solution of Mmt-dTp(Chp)-CAMET-NHBzl (70 mg, 0.072 mmol) in dioxane (4 ml) and H_2O (2.5 ml), was treated with 4-nitrobenzaldehyde oxime (120 mg, 0.72 mmol) and tetramethylguanidine (115 mg, 0.997 mmol). After stirring and keeping the solution for 4 h at 20°, no more educt was seen on TLC. The solution was evaporated to dryness and H_2O removed by coevaporation once with pyridine and once with toluene. The residue was dissolved in the least volume of CMT, applied to a prep. TLC plate (sil; 2 mm thick, 20 × 20 cm) and chromatographed with CMT: 47 mg (68%) of chromatographically pure salt Mmt-dTp-CAMET-NHBzl·NEt_3; R_f 0.4 (sil, CMT).

This triethylammonium salt (2.7 mg, 0.0028 mmol) was dissolved in dioxane (150 µl) and treated with a solution of $NaIO_4$ (12 mg, 0.056 mmol) in H_2O (150 µl). After 40 min at 20°, the solvent was evaporated and H_2O removed by repeated co-evaporation with pyridine. The residue was finally dissolved in pyridine (200 µl) and treated with 200 µl of a solution of Na (1.4 g) in MeOH (15 ml; 4.06M). After 15 h at 20°, a few ml of H_2O were added, and the pH of the solution was adjusted to *ca.* 7 by adding a small amount of *Dowex 50 WX* (100–200 mesh, in the protonated form). Then, an equal volume of dioxane was added, which produced a clear solution that was separated from the resin beads. The filtrate was evaporated down to a volume of about 1 ml; AcOH (4 ml) was added to cleave the 4-methoxytrityl group from the nucleotide. The reaction yielded, besides thymidine 3'-monophosphate, R_f 0.37 (TLC, cel, BAW 523), only two other spots near the front, corresponding to the two products from the CASET(1)-NHBzl- and the Mmt-protecting groups. Thymidine 3'-monophosphate was isolated by dissolution in H_2O extraction with $CHCl_3$, and chromatography of the aq. phase (30 ml) through a column (40 cm × 1 cm) of *DEAE Sephadex* A 25 that had been equilibrated with triethylammonium hydrogen carbonate buffer (1 mM, pH 7.5) and kept at 4° throughout the experiment. A linear gradient (1 mM to 150 mM) of the same buffer was applied to the column during 13 h at an elution velocity of 70 ml/h. Each fraction contained 13 ml (200 drops), dTp was contained in fractions 65–68. λ_{max} was at 267 nm with a maximal extinction of 0.472. The total volume of the fractions was 51 ml, the extinction coefficient of dTp is 9500; thus, the isolated yield of dTp was 2.53 µmol (90% on the basis of Mmt-dTp-CAMET-NHBzl subjected to oxidation).

5.3. *Oxidation of Mmt-dTp-CAMET-NHBzl with 3-Chloroperbenzoic Acid, Acidolysis, and β-Elimination of dTp.* A solution of Mmt-dTp-CAMET-NHBzl·NEt_3 (4 mg, 4 µmol, see *5.2*) in dioxane (100 µl) was frozen at 0° and treated with a solution of 3-chloroperbenzoic acid (12 mg, 70 µmol) in dioxane (100 µl). After thawing and standing for 1 h at 20°, TLC (sil, CMT) revealed the sulfone spot (R_f 0.19) beside a still considerable amount of the starting material (R_f 0.23); the 3-chlorobenzoic and 3-chloroperbenzoic acids appeared together at R_f 0.38. After 4 h, the educt spot had completely vanished, leaving only the sulfone besides the 3-chlorobenzoic acids.

The Mmt group was removed by acidolysis by adding AcOH (300 µl) to the oxidation mixture. After 3 h, the removal was complete, and the solution was evaporated. The org. solvents were removed by repeated co-evaporation with H_2O. β-Elimination was effected by dissolving the residue in dioxane (200 µl) and adding aq. NaOH (200 µl, 0.2N). After 20 min at 20°, the solution was neutralized and the thymidine 3'-phosphate, obtained in a very clean reaction, isolated and identified as above *(5.2)*.

5.4. *Deprotection of the Triester, Mmt-dTp(Chp)-CAMET-(ethylenediamine-polydimethylacrylamide) to Diester dTp-CAMET-resin, Oxidation to dTp-CASET(2)-resin, and β-Elimination of dTp.* The substituted resin obtained in *4.2* (containing about 0.107 mmol of triester groups Mmt-dTp(Chp)-CAMET-) was washed thoroughly with $CHCl_3$/MeOH 7:3, and treated repeatedly with the same solvent mixture containing TsOH (5%, *w/v*). After a short time, the detritylation was complete (no yellow color) and the resin was washed thoroughly with dioxane/H_2O 1:1.

To hydrolyze the 4-chlorophenyl-ester group, the resin was agitated for 15 h, at 20° in a solution of *p*-nitrobenzaldehyde oxime (686 mg, 4.13 mmol) and tetramethylguanidine (658 mg, 5.69 mmol) in dioxane/H_2O 1:1.

After washing the resin with the same solvent mixture until the filtrate was colorless, it was suspended in a saturated solution (0.5M) of 3-chloroperbenzoic acid (2.589 g, 15 mmol) in dioxane/H_2O 1:1 (30 ml) and agitated at 20° for 15 h. The resin was washed repeatedly (10 times) with dioxane/H_2O 1:1 (10 ml each time), gathered in a sintered glass funnel *(G 2)* and treated for 1 h with dioxane/0.2N NaOH in H_2O 1:1. The mixture was filtered and the filtrate neutralized by adding *Dowex 50 WX* (100–200 mesh, in the protonated form). The filtrate from the *Dowex* resin contained thymidine 3′-phosphate as the only product (yield: 84%, determined spectroscopically). The isolated dTp was indistinguishable from a commercial preparation *(Boehringer)* on TLC (cel), R_f 0.47 (BAW 523), 0.09 (INW), see also *5.2*.

5.5 *Oxidation of Mmt-dTp(Chp)-CAMET-(ethylenediamine polydimethylacrylamide) to Mmt-dTp(Chp)-CASET(1)-resin and β-Elimination of the Diester, Mmt-dTp(Chp)-OH.* The substituted resin obtained in *4.2* (20 mg, containing 0.07 mmol of triester groupe Mmt-dTp(Chp)-CAMET-) was washed with dioxane/H_2O 1:1 and treated with a solution of $NaIO_4$ (200 mg) in the same solvent (2.5 ml). After several at 20°, the resin was collected by filtration and thoroughly washed with dioxane/H_2O 1:1, followed by pyridine. The resin was suspended in pyridine (800 µl) and treated with tetramethylguanidine over night. The mixture was filtered and the resin washed with a small amount of pyridine. The resin then contained no more Mmt-groups (negative color test with $HClO_4$/EtOH 6:4). The filtrate contained Mmt-dTp(Chp)-OH as the only product. It was characterized by TLC (sil) and found to behave like an authentical sample: R_f 0.0 (CM), 0.28 (CMT). The product was condensed with 3′-(*tert*-butyldimethylsilyl)thymidine to obtain the corresponding fully protected dinucleoside monophosphate, Mmt-dTP(Chp)-dT-X (X=*tert*-butyldimethylsilyl; to be published elsewhere). The β-elimination reaction had proceeded to 90% after 6 h and was complete after 15 h.

6. Amino-Acid Derivatives of CASET(2) and Active Esters. – 6.1. *(2-(4-Carboxyphenylsulfonyl)ethyl* N-*(*tert-*Butoxycarbonyl)*-L-*leucinate (Boc·Leu·CASET(2)·OH).* A solution of Boc-Leu-OH (10.97 g, 44 mmol) in DMF (30 ml) was treated with DCC (4.54 g, 22 mmol) at 0°. After ½ h, the dicyclohexylurea was removed by filtration, washed with a small amount of the same solvent, and the combined filtrate added dropwise to a cold (0°) solution of CASET(2) (4.6 g, 20 mmol) in abs. pyridine. After 1 h at 0° and 15 h at r.t., the mixture was evaporated at 0.01–0.1 Torr. The residue was dissolved in AcOEt/(i-Pr)$_2$O 1:1, acidified with 1N HCl, washed with H_2O and sat. NaCl, dried over $MgSO_4$, and partially evaporated. The product was precipitated as an oil by adding petroleum ether; it solidified after a few h at 0°. Recrystallization from AcOEt/(i-Pr)$_2$O 2:8 with pentane: 7 g (79%) of a slightly impure product. Recrystallization from 80 ml of the same solvent mixture afforded 5.4 g (60.8%) of pure Boc-Leu-CASET(2)-OH; m.p. 112–123°; $[\alpha]_D^{25} = -11.68°$ (*c* = 1.25, EtOH). TLC (sil, CMA 9553): R_f 0.4. Anal. calc. for $C_{20}H_{29}O_8S$ (443.52): C 54.17, H 6.59, N 3.16, S 7.23; found: C 54.49, H 6.78, N 3.56, S 7.40.

6.2. *2-[4-(2,4,5-Trichlorophenoxycarbonyl)phenylsulfonyl]ethyl* N-*(*tert-*Butoxycarbonyl)*-L-*leucinate (Boc-Leu-CASET(2)-OTcp).* A cold (0°) solution of Boc-Leu-CASET(2)-OH (2.217 g, 5 mmol) and 2,4,5-trichlorophenol (1.48 g, 7.5 mmol) in CH_2Cl_2 (75 ml) was treated with solid DCC (1.135 g, 5.5 mmol) and left to react over night at r.t. The precipitated dicyclohexylurea was removed by filtration, the filtrate evaporated, and the residue dissolved in (i-Pr)$_2$O and treated with glacial AcOH (50 µl). After 2 h, the dicyclohexylurea was again filtered off, the filtrate partially evaporated, and the product precipitated with petroleum ether. (The mother liquor contained essentially 2,4,5-trichlorophenol.) The precipitate was dissolved in warm (i-Pr)$_2$O. Pure Boc-Leu-CASET-OTcp crystallized upon addition of pentane: 2.4 g (77%); m.p. 66–67°; $[\alpha]_D^{25} = +3.82°$ (*c* = 1.31, EtOH). TLC (sil, CMA 9553): R_f 0.74. Anal. calc. for $C_{26}H_{30}Cl_3NO_8S$ (622.95): C 50.12, H 4.85, N 2.25, S 5.15; found: C 49.78, H 4.85, N 2.17, S 5.40.

Boc-Leu-CASET(2)-OTcp was also obtained by reacting the symmetrical anhydride prepared from Boc-Leu-OH (1.5 g) in AcOEt (see *6.1*) with H-CASET(2)-OTcp (1.07 g; see *2.2*) in dry pyridine, as above.

6.3. *2-(4-Carboxyphenylsulfonyl)ethyl* N-*(*tert-*Butoxycarbonyl)glycinate (Boc-Gly-CASET(2)-OH).* The symmetrical *N*-(*tert*-Butoxycarbonyl)glycine anhydride was prepared from Boc-Gly-OH (3.85 g, 22 mmol) and

DCC (2.27 g, 11 mmol) as described in *6.1* and reacted with CASET(2) (2.30 g, 10 mmol) in abs. pyridine (10 ml): 2.0 g (52%) of pure Boc-Gly-CASET(2)-OH as colorless crystals from AcOEt/petroleum ether; m.p. 109–111° TLC (sil): R_f 0.19 (CMA 9553), 0.79 (CM). Anal. calc. for $C_{16}H_{21}NO_8S$ (387.42): C 49.60, H 5.46, N 3.62, S 8.28; found: C 49.36, H 5.36, N 3.33, S 8.49.

6.4. *2-[4-(2,4,5-Trichlorophenoxycarbonyl)phenylsulfonyl]ethyl* N-(tert-*Butoxycarbonyl)glycinate (Boc-Gly-CASET(2)-OTcp)*. Obtained as long, colorless needles (1.7 g, 77.7%; from AcOEt/$(i\text{-Pr})_2O$ or AcOEt/petroleum ether) from Boc-Gly-CASET(2)-OH (1.5 g, 3.9 mmol), 2,4,6-trichlorophenol (1.16 g, 5.9 mmol) and DCC (0.9 g, 4.3 mmol) as described for *6.2;* m.p. 131–133°. TLC (sil, CMA 9553): R_f 0.82. Anal. calc. for $C_{22}H_{22}Cl_3NO_8S$ (566.85): C 46.61, H 3.91, N 2.47, S 5.66, Cl 18.77; found: C 46.90, H 4.11, N 2.62, S 5.65, Cl 18.59.

6.5. *2-(4-Carboxyphenylsulfonyl)ethyl* N-(tert-*Butoxycarbonyl)*-L-*phenylalaninate (Boc-Phe-CASET(2)-OH)*. Obtained as colorless crystals (3.3 g, 69,2%; from i-PrOH/$(i\text{-Pr})_2O$) from Boc-Phe-OH (5.84 g, 22 mmol), DCC (2.27 g, 0.11 mmol), and CASET(2) (2.3 g, 10 mmol) as described in *6.1*. M.p. 111–112°; $[\alpha]_D^{25} = +11.21°$ (c = 1.41, EtOH). TLC (sil): R_f 0.31 (CMA 9553), 0.79 (CM). Anal. calc. for $C_{23}H_{27}NO_8S$ (477.54): C 57.85, H 5.70, N 2.93, S 6.72; found: C 57,97, H 5.71, N 2.86, S 6.81.

6.6. *2-[4-(*N-*Succinimidooxycarbonyl)phenylsulfonyl]ethyl* N-(tert-*Butoxycarbonyl)*-L-*phenylalaninate (Boc-Phe-CASET(2)-ONSu)*. Obtained as colorless crystals (1.5 g, 83%, from AcOEt/$(i\text{-Pr})_2O$) from Boc-Phe-CASET(2)-OH (1.50 g, 3.14 mmol), DCC (720 mg, 3.48 mmol), and *N*-hydroxysuccinimide (400 mg, 3.48 mmol) in dry CH_2Cl_2 (20 ml) as described in 6.2. M.p. 83–84°. TLC (sil, CMA 9553): R_f 0.48. Anal. calc. for $C_{27}H_{30}N_2O_{10}S$ (574.61): C 56.44, H 5.26, N 4.88, S 5.58; found: C 56.21, H 5.49, N 4.72, S 5.45.

6.7. *2-(4-Carboxyphenylsulfonyl)ethyl* N-(tert-*Butoxycarbonyl)*-D-*alaninate (Boc-ala-CASET(2)-OH)*. Obtained as colorless crystals (3.2 g, 79,8%; from AcOEt/petroleum ether) from Boc-D-Ala-OH (4.16 g, 22 mmol), DCC (2.27 g, 11 mmol), and CASET(2) (2.30 g, 10 mmol) as described in *6.1*. M.p. 135–136°; $[\alpha]_D^{25} = +16.67°$ (c = 1.02, EtOH); TLC (sil): R_f 0.24 (CMA 9553), 0.79 (CM 91). Anal. calc. for $C_{17}H_{23}NO_8S$ (401.46): C 50.86, H 5.78, N 3.49, S 7.99; found: C 50.94, H 5.74, N 3.40, S 8.09.

6.8. *2-[4-(2,4,6-Trichlorophenoxycarbonyl)phenylsulfonyl]ethyl* N-(tert-*Butoxycarbonyl)*-D-*alaninate (Boc-ala-CASET(2)-OTcp)*. Obtained as colorless crystals (2.8 g, 96,5%; from AcOEt/$(i\text{-Pr})_2O$) from Boc-D-Ala-CASET(2)-OH (2.0 g, 5 mmol), 2,4,5-trichlorophenol (1.5 g, 7.5 mmol) and DCC (1.14 g, 5.5 mmol) as described in *6.2*. M.p. 137–138°; $[\alpha]_D^{25} = +2.29°$ (c = 1.44, EtOH). TLC (sil, CMA 9553): R_f 0.82. Anal. calc. for $C_{23}H_{24}Cl_3O_8NS$ (580.90): C 47.55, H 4.16, N 2.41, S 5.52, Cl 18.31; found: C 47.83, H 4.27, N 2.43, S 5.59, Cl 18.02.

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