Tetrahedron Letters, Vol.32, No.52, pp 7723-7726, 1991
Printed in Great Britain

0040-4039/91 $3.00 + (
Pergamon Press plc

Synthesis of an Amino Acid Based Phosphodiester Linkage Containing Cryptand.

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Abstract: The synthesis of an amino acid based phosphodiester linkage containing cryptand is described. The macrocyclic ring is constructed using Boc-*L*-Ser(OBn)-OH, Boc-*D*-Ser(OBn)-OH, diethylene glycol and ethylene dioxydiacetic acid. The phosphodiester linkage was introduced employing 4-chlorobenzyl dichlorophosphite leading to the cryptand

In a recent communication[1], we described the synthesis of a cyclic phosphopeptide containing a phosphodiester linkage. We were intrigued by the possibility that a phosphodiester linkage between two hydroxyl amino acids might serve a similar purpose as a disulfide linkage, i e to retain or stabilize the structure of a protein The synthesis of cyclic phosphopeptides[1,2] aroused our interest in the possibility to employ the phosphodiester linkage as a constraint in the construction of semi-peptide phosphate-containing macrocycles

Macrocyclic structures, which contain a phosphate group, have not been widely studied[1-3]. The presence of a "P=O" moiety could lead to interesting receptor molecules, which might be able to bind metal ions and organic cations[3,4].

So far the phosphate moiety has not been used as a possible recognition unit in the construction of macrocyclic receptor molecules In contradistinction, the carboxylic acid moiety has been extensively studied as a recognition unit in the elegant receptor molecules of Rebek *et al.*[5].

As an approach towards the design and synthesis of phosphodiester linkage containing receptor molecules, we describe here the first preparation of a phosphodiester linkage containing, amino acid based[6], cryptand **11** and summarize some preliminary data on its host-guest complexation behavior

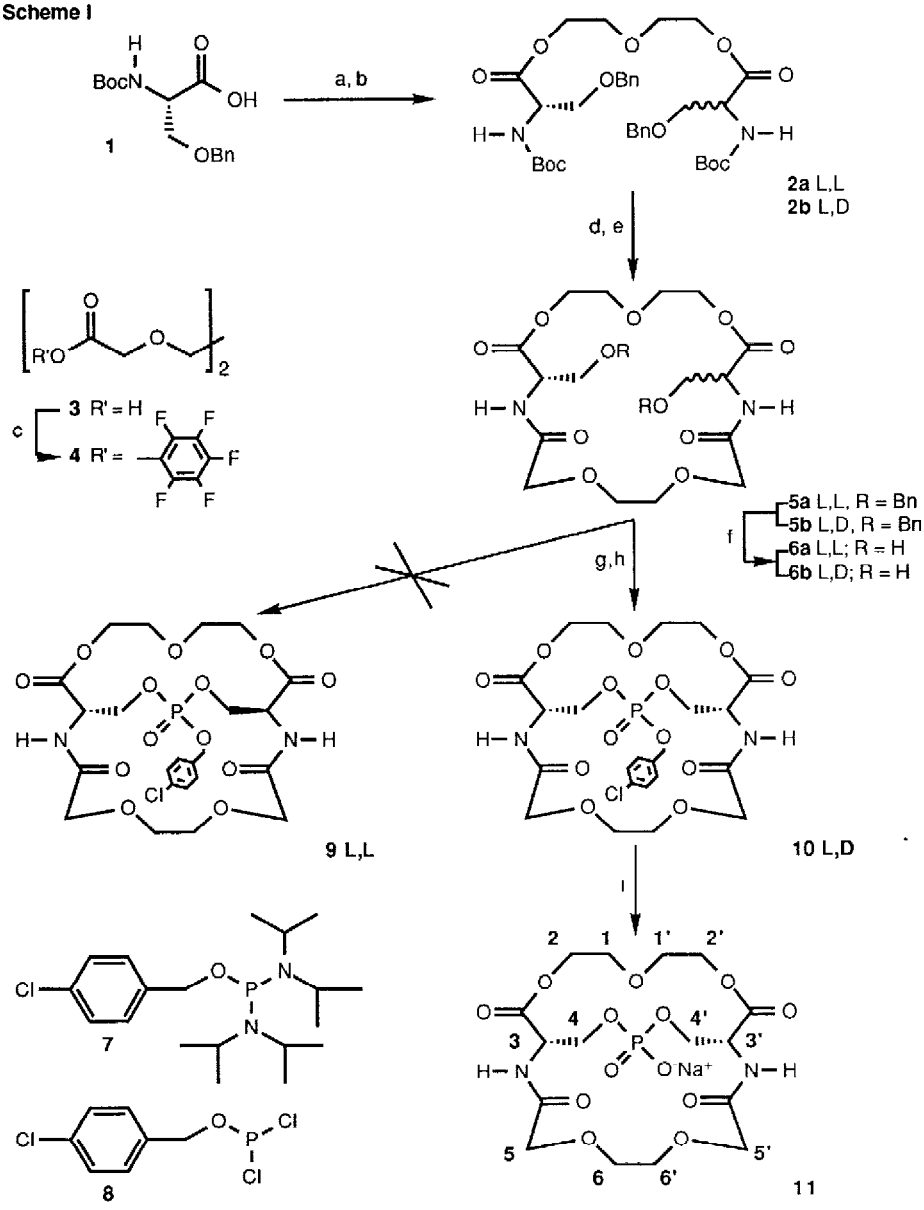
We first attempted to synthesize the protected L,L-cryptand **9** Starting from commercially available Boc-L-Ser(OBn)-OH (**1**), the L,L-precursor **6a** was prepared in five steps (scheme 1) Unfortunately, we were unable to introduce the phosphate linkage leading to the protected L,L-cryptand **9**, using bis(*N,N*-diisopropylamino) 4-chlorobenzyl phosphoramidite **7**[1] Even attempts using the more reactive phosphitylating agent 4-chlorobenzyl dichlorophosphite **8**[7] failed. In trying to explain this finding, we reasoned that there might be some kind of geometrical constraint preventing the formation of the phosphate linkage. The most obvious cause of geometrical constraint (vide infra) is the stereochemistry of one of the serine residues.

Indeed, when the corresponding L,D-precursor **6b** - prepared analogously to the L,L-precursor (vide supra) - was subjected to phosphitylation with the reactive phosphitylating agent **8**[7], we were able to obtain the protected L,D-cryptand **10** in 31% yield Interestingly, it was not possible to obtain this compound using the less reactive phosphitylating agent bis(*N,N*-diisopropylamino) 4-chlorobenzyl phosphoramidite **7**, although this reagent has been used previously with success to prepare cyclic phosphopeptides[1,2]

The diastereomeric phosphotriesters of the protected L,D-cryptand **10** were formed in approximately equal amounts and could not be separated by short column chromatography[8].

Hydrogenolysis of the 4-chlorobenzyl group under buffered[9] conditions afforded the sodium salt of the L,D-cryptand **11**[10]

Scheme I



(a) Diethylene glycol (10 equiv.), DMAP, CH_2Cl_2, DCC (80%). (b) Boc-L-Ser(OBn)-OH (**a**) or Boc-D-Ser(OBn)-OH (**b**), DMAP, CH_2Cl_2, DCC (79% and 80% resp). (c) Pentafluorophenol, dioxan, DCC (77%) (d) TFA, CH_2Cl_2. (e) **4**, 4-methylmorpholine, THF (step d + step e 35% and 32% resp). (f) *t*-BuOH, H_2O, Pd/C, H_2 (100%). (g) **8**, *N,N*-diisopropylethylamine, CH_2Cl_2 (h) *m*-CPBA (step g + step h 31%) (i) 1 *t*-BuOH, H_2O, Pd/C, NaOAc (1.1 equiv.), H_2, 2. Sephadex LH-20 (90%).

As to an explanation why phosphitylation leading to the corresponding L,L-cryptand had failed, we took recourse to computer assisted molecular modeling studies[11]. Inspection of the global minimum of the L,L-precursor **6a** as well as conformations lying within a 10 kJ window showed that the distance between both oxygens of the hydroxyl groups varies between 5.1 and 7.7 Å, which has to be reduced to a distance of ca. 2 6 Å in a model of the protected L,L-cryptand **9**. Simultaneously, in order to form the protected L,L-cryptand, it appears that the L,L-precursor has to undergo extensive unfolding. Rotation of notably the α-C-N(H) bonds, in order to move one of the hydroxyl groups through the cavity of the macrocycle, is necessary to approach the geometry of the L,L-cryptand structure. In contradistinction, the distance between both oxygens of the hydroxyl groups in the global minimum the L,D-precursor **6b** and conformations lying within a 10 kJ window varies between 2.7 and 6 4 Å, which has to be reduced to ca 2.6 Å in the protected L,D-cryptand **10** The geometry of the global minimum of the L,D-precursor already resembles the geometry of the L,D-cryptand structure (see figure 1), thus facilitating the formation of the latter The necessity for a smaller reduction of the O-O distance in the L,D-cryptand is also reflected in a more favorable enthalpy of formation of the protected L,D-cryptand[12]; -14.9 kJ/mol as compared to -2 9 kJ/mol for formation of the L,L-cryptand

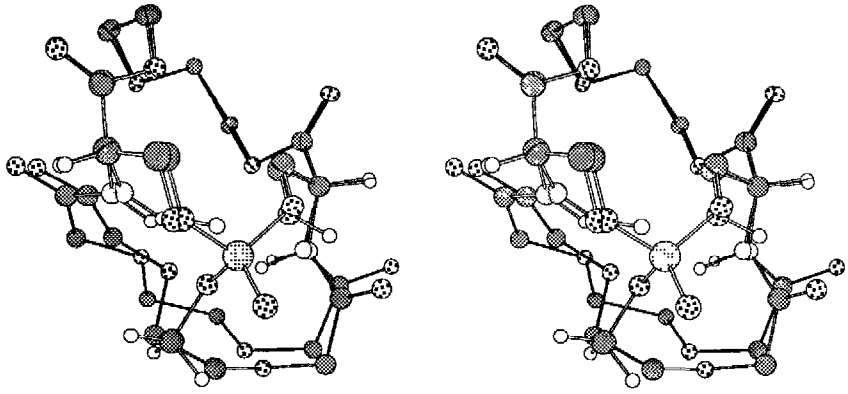


Figure 1. Stereoview of a superimposition of the global minima of L,D-precursor **6b** and L,D-cryptand **10**

In conclusion, the described method for the introduction of a phosphodiester linkage leading to a phosphocryptand, provides a synthetic route to amino acid based phosphodiester linkage containing cryptands. An acid functionality, the phosphodiester, can be introduced in this type of molecules in a convergent manner. Host-guest complexation behavior will be studied using L,D-cryptand **10** and more lipophilic cryptand derivatives. In addition, changes in the structure of the cryptand, i.e. changing the amino acids, the diethylene glycol part or the ethylene dioxydiacetic acid part, which may lead to different receptor behavior, are under present investigation.

Acknowledgement

We thank C. Erkelens for recording some of the NMR spectra and R. Fokkens of the Institute of Mass Spectoscopy of the University of Amsterdam for recording the FAB-mass spectra.

References and Notes

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2. Manuscript in preparation.

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4. The "P=O" moiety in, e.g., triphenylphosphine is a good proton acceptor and can form complexes with a variety of organic bases; see e g.. Etter, M.C.; Baures, P W *J. Am. Chem. Soc.* **1988**, *110*, 639 and references cited therein.

5. For reviews see: Rebek, J., Jr. Recent Progress in Molecular Recognition, *Topics in Current Chemistry* **1988**, *149*, 189; Rebek, J., Jr. *Pure & Appl. Chem.* **1989**, *61*, 1517, Rebek, J , Jr. *Heterocycles* **1990**, *30*, 707.

6 For examples of amino acid based receptor molecules see: Kellogg, R.M. Bioorganic Modelling - Stereoselective Reactions with Chiral Neutral Ligand Complexes as Model Systems for Enzyme Catalysis, *Topics in Current Chemistry* **1982**, *101*, 111; Kellogg, R M *Angew. Chem Int. Ed. Engl.* **1984**, *23*, 782; Kaptein, B ; Kellogg, R.M.; Van Bolhuis, F. *Recl. Trav. Chim. Pays-Bas* **1990**, *109*, 388, Kilburn, J.D.; McKenzie, A R ; Still, W C. *J. Am. Chem. Soc.* **1988**, *110*, 1307; Sanderson, P E.; Kilburn, J D.; Still, W.C *ibid.* **1989**, *111*, 8314, Hong, J -I ; Namgoong, S.K., Bernardi, A.; Still, W.C. *ibid.* **1991**, *113*, 5111.

7 The 4-chlorobenzyl dichlorophosphite was prepared from PCl_3 analogous to the procedure described for benzyl dichlorophosphite (Bannwarth, W.; Trzeciak, A. *Helv. Chim. Acta* **1987**, *70*, 175). This reagent was also prepared by Caruthers *et al* (Caruthers, M.H.; Kierzek, R.; Tang, J.Y.; In. *Biophosphates and Their Analogues - Synthesis, Structure, Metabolism and Activity*; Bruzik, K.S., Stec, W J , Eds.; Elsevier Science Publishers· Amsterdam, 1987; p 3) in a different manner

8. Separation of both diastereoisomers using HPLC chromatography with an CN-column however, was possible. The diastereomeric ratio was 11:9, estimated using ^{1}H NMR-data which is in agreement with the estimation by HPLC chromatography.

9. De Bont, H.B A ; Van Boom, J.H., Liskamp, R M J *Recl Trav. Chim. Pays-Bas* **1990**, *109*, 27

10. ^{1}H NMR spectra were recorded on a Bruker WM 300 MHz apparatus, ^{13}C and ^{31}P NMR specta were measured on a Bruker MSL-400 spectrometer. NMR data of L,D-cryptand **11** (sodium salt)· ^{1}H NMR (CD_3OD) d 3.54-3 68 (m, (1+1')), 3.78-3.87 (m, (6+6')), 3 99 (d, $(5+5')_a$, $J_{HH,gem}$ = 15.8 Hz), 4.17 (d, $(5+5')_b$), 4.21-4.39 (m, (2+2'), (4+4')),4.58 (dd, (3+3'), $J_{HH,vic}$ = 3.3 Hz, $J_{HH,vic}$ = 5.5 Hz); ^{13}C NMR (CD_3OD) 56.0 ((3+3')), 65.6 ((2+2')), 65.6 ((4+4'), J_{CP} = 4.8 Hz), 69.9 ((1+1')), 71.6 ((5+5')), 71.9 ((6+6')), 171 0, 173 1 (2 C(O)); ^{31}P NMR 4.56. FAB-mass spectrum *m/z* M(sodium salt) + Na^+ 529.7.

11 Global minima were found with the Monte Carlo-method (Chang, G ; Guida, W.C., Still, W C. *J. Am. Chem. Soc.* **1989**, *111*, 4379) present in BATCHMIN of MacroModel (Mohamadi, F.; Richards, N G.; Guida, W.C ; Liskamp, R.; Lipton, M.; Caufield, G.; Chang, G.; Hendrickson, T.; Still, W C. *J. Comp. Chem.* **1990**, *11*, 440.). The AMBER force field (Weiner, S.J., Kollman, P.A.; Case, D.A.; Singh, U.C.; Ghio, C., Alogona, G., Profeta, S , Weiner, P *J. Am. Chem. Soc.* **1984**, *106*, 765; Weiner, S.J.; Kollman, P A ; Nguyen, D.T , Case, D A. *J. Comp. Chem.* **1986**, *7*, 230.) was used in MacroModel

12. To facilitate calculations the 4-chlorobenzyl group was replaced by a methyl.

(Received in UK 8 October 1991)