61. Lithium-Salt Effects in Peptide Synthesis

Part I

Conditions for the Use of Lithium-Salts in Coupling Reactions

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The influence of Li-salts on the course of peptide-coupling reactions was investigated. As a model for segment couplings, Ac-Phe-OH was coupled to HCl·H-Ala-OMe using the mixed anhydride, DCCl, DCCl/HOBt, BOP-*Castro* and TBTU-*Knorr* methods. As a model for stepwise synthesis Z-Phe-OH was coupled with HCl·Ala-O(*t*-Bu), using symmetrical anhydrides and active esters. The effects of salt additives such as LiCl, LiBr, $LiClO_4$, and $ZnCl_2$ on yields, side-product formation, racemisation, and reaction rates are reported.

Introduction. – Limited solubility of peptide intermediates in the reaction media [1] [2] can be a serious obstacle to peptide synthesis in solution [2–5]. Similarly, poor solvation of peptide-resin intermediates can lead to very slow and, therefore, often incomplete reactions in solid-phase peptide synthesis (SPPS) [6–9]. Despite the widespread use of strongly solvating polar solvents, *e.g.*, dimethylformamide (DMF) or dimethylacetamide (DMA) for peptide synthesis in solution as well as in solid phase, solubility and solvation problems are still persisting. Previously, it has been reported that the solubility of peptides of widely varying structure is greatly enhanced in ether-type solvents[2]) by the addition of inorganic salts[3])[4]) [11]. The basis for this solubilising effect probably lies in the potential of peptides for forming complexes with group-I and -II cations. Such complexes have been shown for amino acids and peptides in crystals [12–14] as well as in solution [11] [15–19]. In addition to a solubilising effect on the reactants, Li-salts may also modify the mechanism and course of a chemical reaction[5]). The influence of Li-salts on standard peptide reactions has not yet been studied. However, before Li-salts can be used rationally as solubilising agents in general peptide chemistry, it should first be learned which and to what extent peptide-forming reactions are tolerant to such additives. Otherwise we

[1]) Part of the projected Ph. D. thesis of *A. T.*, ETH Zürich.

[2]) Tetrahydrofuran, dimethoxyethane, or polyethyleneglycol 200.

[3]) LiCl, LiBr, LiI, $LiBF_4$, $LiClO_4$, NaI, $MgBr_2$, $CaBr_2$, $ZnCl_2$ and titanates ($Ti(OEt)_4$, $Ti(OCHMe_2)_4$).

[4]) In independant work, *Morii* and *Ichimura* reported recently about peptide-solubility enhancement in DMF upon addition of 4 to 7% LiCl [10].

[5]) Some examples are mentioned in [11].

may trade a gain in solubility for a decrease in reactivity or for increased side reactions. Here, we report on the effects of certain salts (LiCl, LiBr, $LiClO_4$, $LiBF_4$) on yields, by-product formation, racemisation, and kinetics of peptide-coupling reactions in solution. As a model, we chose the dipeptide Phe-Ala for its simplicity and ease of analysis in the *Halpern-Weinstein* racemisation test (^{1}H-NMR) [20]. By selecting for this study a model of which the peptide components are fully soluble, we were able to assess the effect of a given additive on the coupling reaction separately from its solubilising effect on the reaction substrates[6]).

Results. – Applying the *mixed-anhydride* method (*Schema, Method A*), we noticed no racemisation under the standard reaction conditions using 4-methylmorpholine (NMM) in tetrahydrofuran (THF) at −20° for the coupling of Ac-Phe-OH (**1**) to HCl·H-Ala-OMe (**2**). Besides the main product Ac-Phe-Ala-OMe (**3**), 6% of the urethane side product **4**[7]) were observed (see *Table 1*). The addition of Li-salts did not promote racemisation but led to an increase of urethane **4** relative to the product **3**. The extent of

Table 1. *Coupling of Ac-Phe-OH* (**1**) *and HCl·H-Ala-OMe* (**2**) *by the Mixed-Anhydride Method* (using isobutyl chloroformate) *in THF to Give Ac-Phe-Ala-OMe* (**3**; L,L) *and Its Epimer* (D,L)

Added salt [equiv.]	Temp. [°]	Base	Reaction time [h]	Yield [%][a])	Epimer ratio L,L/D,L[b])	Product ratio **3**/**4**[c])
none	−20 to 25	NMM	21	75	≥ 97:3	94:6
10 LiCl	−20 to 25	NMM	21	77	≥ 97:3	61:39
10 $LiClO_4$	−20 to 25	NMM	21	81	≥ 97:3	70:30
10 $LiBF_4$	−20 to 25	NMM	21	63	≥ 97:3	89:11
none	25	Et_3N	16	44	36:64	≥ 97:3
5 LiCl	25	Et_3N	6.5	78	55:45	70:30
5 LiCl[d])	25	Et_3N	16	44	72:28	37:63
5 $LiClO_4$[d])	25	Et_3N	16	65	71:29	70:30

[a]) Yield determined as crude product isolated.
[b]) Ratio of the epimers determined by NMR analysis.
[c]) Ratio of dipeptide **3** to urethane **4** determined by NMR analysis.
[d]) Salt added to **1** prior to the formation of the mixed anhydride (in the other cases, the salts were added to the solution of the amine before coupling).

urethane formation decreased from LiCl to $LiClO_4$ to $LiBF_4$. Use of Et_3N as base[8]) at room temperature without salt additives resulted in much racemisation. This could be reduced by the addition of LiCl or $LiClO_4$ to the coupling mixture. Both salts increased formation of the by-product urethane in this reaction.

Another well known coupling method is the *dicyclohexylcarbodiimide* (DCCI; **5**) method (*Scheme, Method B*) with or without addition of 1-hydroxy-1*H*-benzotriazole[9]) (HOBt; **6**). In THF using DCCI alone, we found a much lower yield in the presence of

[6]) For an extension of this study to the solid-phase synthesis of peptides, see the accompanying paper [21].
[7]) The formation of **4** is caused by a 'wrong' attack of the amine on the mixed anhydride and is a known side reaction in mixed-anhydride couplings [22].
[8]) Et_3N is known to cause more racemisation and formation of **4** than NMM: Et_3N > NMM > 1-methylpiperidine [23] [24].
[9]) HOBt is known to suppress the formation of *N*-acylurea **7** and to minimize racemisation in DCCI coupling reactions [25].

$LiClO_4$ and only traces of product with LiCl (see *Table 2*). For DCCI/HOBt couplings, we found only a moderate yield when LiCl was used as an additive and observed some racemisation. In DMF using DCCI alone, racemisation was comparable in the presence or absence of Li-salts, but yields were generally smaller in the presene of Li-salts. For

Scheme. *Coupling Methods Tested for the Effect of Salt Additives*

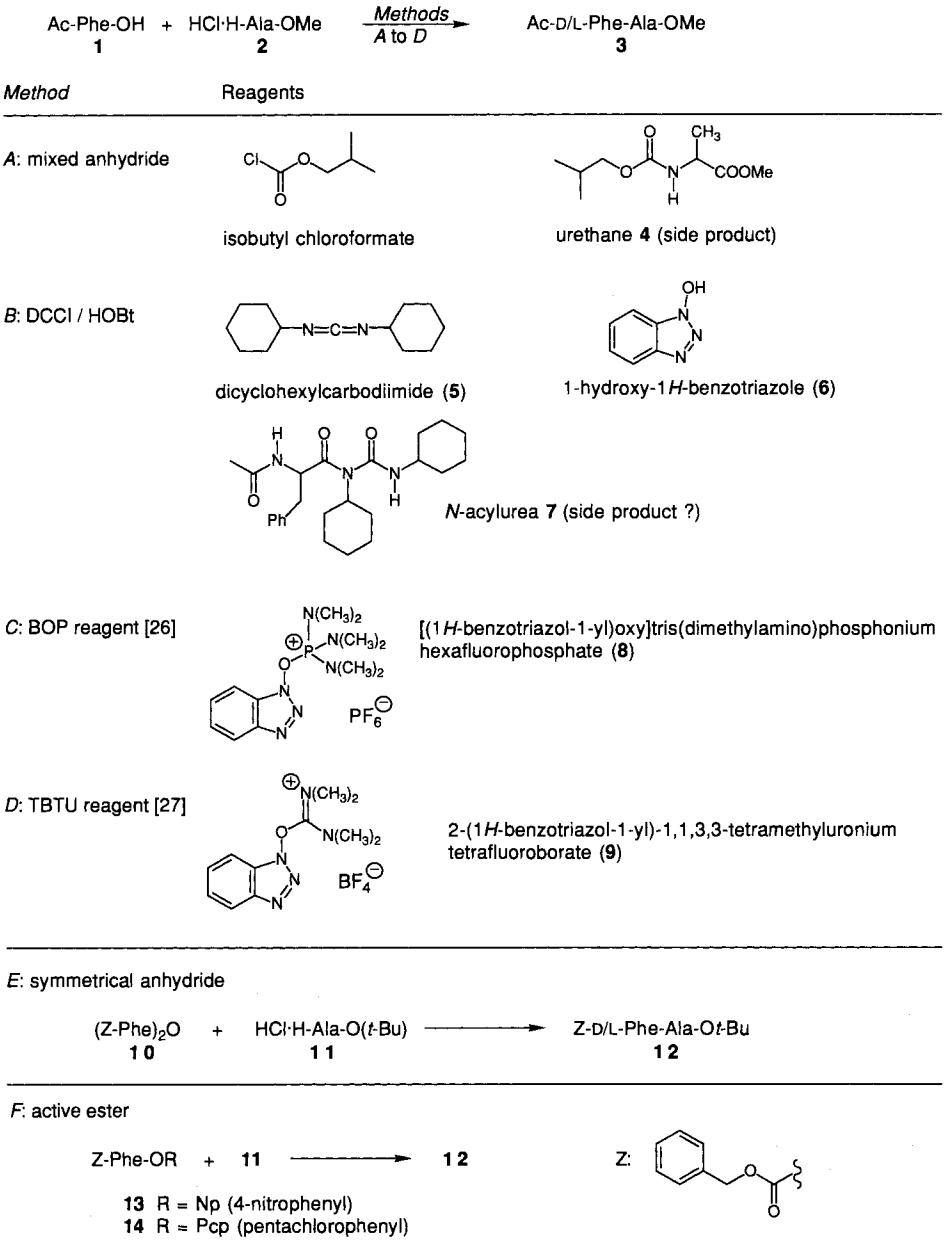

Table 2. *Coupling of Ac-Phe-OH* (**1**) *and HCl·H-Ala-OMe*[a]) (**2**) *by the DCCI* (**5**) *and DCCI* (**5**)*/HOBt* (**6**) *Method to Give Ac-Phe-Ala-OMe* (**3**; L,L) *and Its Epimer* (D,L)

Method	Added salt	Solvent	Temp. [°]	Reaction time [h]	Yield [%][b])	Epimer ratio (L,L/D,L)
5	none	THF	25	6	66	75:25
5	0.5M LiCl	THF	25	6	traces	–
5	0.5M $LiClO_4$	THF	25	6	25	64:36[d])
5	none	DMF	25	3	41	63:37
5	0.5M LiCl	DMF	25	3	21	69:31
5	0.5M $LiClO_4$	DMF	25	3	24	64:36
5/6	none	THF	−20 to 25	*ca.* 48	89	⩾97:3
5/6	0.5M LiCl	THF	−20 to 25	*ca.* 48	27	91:9
5/6	none	DMF	−22 to 25	20	62	96:4
5/6	6 equiv. LiCl	DMF	−22 to 25	20	65	88:12
5/6	6 equiv. $LiBF_4$	DMF	−22 to 25	20	72	96:4

[a]) 1 equiv. of NMM added.
[b]) Isolated product after chromatographic separation.
[c]) As in *Table 1*.
[d]) Slightly impure as judged from the ^{1}H-NMR spectrum.

DCCI/HOBt couplings in DMF, yields were similar with and without added LiCl or $LiBF_4$. While $LiBF_4$ did not seem to promote racemisation, the addition of LiCl led to a somewhat higher degree of racemisation when compared to the salt-free conditions.

Excellent yields and short coupling times were observed with BOP reagent (**8**; *Castro* and coworkers [26]) and TBTU reagent (**9**, *Knorr* and coworkers [27]) (*Table 3* and *Scheme, Method C* and *D*, respectively). Yields were unaffected by the addition of LiCl or $LiBF_4$, but racemisation increased upon addition of weakly nucleophilic and even more so with highly nucleophilic salts. Considerable racemisation was also observed, when no salts were added (*Table 3*). The use of NMM instead of Et_3N could possibly reduce this effect.

Table 3. *Coupling of Ac-Phe-OH* (**1**) *and HCl·H-Ala-OMe* (**2**) *by* BOP *Reagent* (**8**) *or TBTU Reagent* (**9**)[a]) *to Give Ac-Phe-Ala-OMe* (**3**; L,L) *and Its Epimer* (D,L)

Method	Added salt [equiv.]	Reaction time [h]	Yield [%][b])	Epimer ratio L,L/D,L[b])
8	none	3.5	93	87:13
8	6 LiCl	3.5	92	73:27
8	6 $LiBF_4$	3.5	92	82:18
9	none	2	86	88:12
9	6 LiCl	2	84	77:23
9	6 $LiBF_4$	2	81	84:16

[a]) Reactions in DMF at room temperature in the presence of 2 equiv. of Et_3N.
[b]) As in *Table 1*.

Coupling methods using preactivation (see the *Scheme, Methods E* and *F*) generally gave excellent yields of pure products, and no racemisation was detectable. Preformed *symmetrical anhydrides* $(Z\text{-Phe})_2O$ (**10**) or *active esters* Z-Phe-ONp (**13**) and Z-Phe-OPcp (**14**) were coupled with HCl·H-Ala-O(*t*-Bu) (**11**) to give Z-Phe-Ala-O(*t*-Bu) (**12**). As

Table 4. *Coupling of Preformed Symmetrical Anhydride (Z-Phe)$_2$O* (**10**) *or Active Ester Z-Phe-ONp* (**13**) *and Z-Phe-OPcp* (**14**) *with HCl·H-Ala-O*(t-*Bu*) (**11**) *to Give Z-Phe-Ala*(t-*Bu*) (**12**; L,L) *and Its Epimer* (D,L)

Reagent	Added salt [equiv.]	Solvent	Temp. [°]	Base	Reaction time [h]	Yield [%][a])	Epimer ratio L,L/D,L[a])
10	none	DMF	−20 to 25	Et_3N	96	94	⩾97:3
10	5 LiCl	DMF	−20 to 25	Et_3N	96	94	⩾97:3
13	none	THF	25	NMM	24	88	⩾97:3
13	6 LiCl	THF	25	NMM	24	91	⩾97:3
13	none	DMF	−10	NMM	3.5	39[b])	⩾97:3
13	6 LiCl	DMF	−10	NMM	3.5	44[b])	⩾97:3
14	none	DMF	−40 to 25	NMM	18	87	⩾97:3
14	6 LiCl	DMF	−40 to 25	NMM	18	92	⩾97:3
14	6 $LiBF_4$	DMF	−40 to 25	NMM	18	89	⩾97:3

[a]) As in *Table 1*. [b]) Based on the ratio of **12**–**13** in ^{1}H-NMR.

shown in *Table 4*, coupling yields were not affected by the addition of Li-salts such as LiCl or $LiBF_4$, and we never detected any racemisation.

What is the effect of salt additives on the reaction rate of a coupling reaction? For this study, we chose the active-ester coupling of **13** with **11** (see *Scheme*)[10]. As shown in the *Figure*, LiCl accelerates the reaction in DMF (*a*) and in *N*-methylpyrrolidin-2-one (NMP, *b*), whereas in THF no effect was observed (*c*). Interestingly, LiCl in THF activated weakly active phenyl esters, albeit complete conversion could be achieved only under drastic conditions (THF/reflux and/or reaction times of up to five days, see the *Fig., d*). Additionally, we tested some other salts (curves not shown). LiBr accelerates the reaction as well as LiCl. $LiBF_4$ and $LiClO_4$ showed no effect in DMF at room temperature, whereas with KSCN and $NaClO_4$, we noted a slight rate decrease when compared to salt-free conditions. With $ZnCl_2$, only very small conversions were detected.

Discussion. – With a view to applications in segment couplings, we first chose a *N*-acetyl-protected amino acid as a model for peptide segments which are prone to epimerisation ('racemisation'). Among the coupling methods tested (mixed anhydride, DCCI, DCCI/HOBt), we observed several negative effects of salt additives on the formation of by-products or racemisation during coupling reactions. According to our results, only few conditions using salt additives can be recommended for use in segment coupling.

For the *mixed-anhydride* method, low temperature and a non-nucleophilic Li-salt such as $LiBF_4$ are required in order to minimize the formation of urethane side product **4**. It seems that the Li-salt in a mixed-anhydride complex to some extent activates the 'wrong' CO group. Under forcing conditions such as Et_3N at room temperature, Li-salts decrease racemisation in these coupling reactions. The ratio of the epimers L,L/D,L (36:64) indicates, that the activated D-Phe derivative (formed by racemisation during activation) reacted significantly faster than the L-Phe derivative.

The *DCCI* method with Li-salts can only be recommended in the presence of HOBt in DMF as solvent. Among the salts tested, $LiBF_4$ gave the least racemisation. With DCCI alone, or using THF as solvent, poor yields were observed, although the results obtained in DMF are not fully comparable to those in THF, because different amounts of HOBt

[10]) The ratio between starting material **13** and product dipeptide **12** could easily be determined by HPLC on aliquots of the reaction mixture.

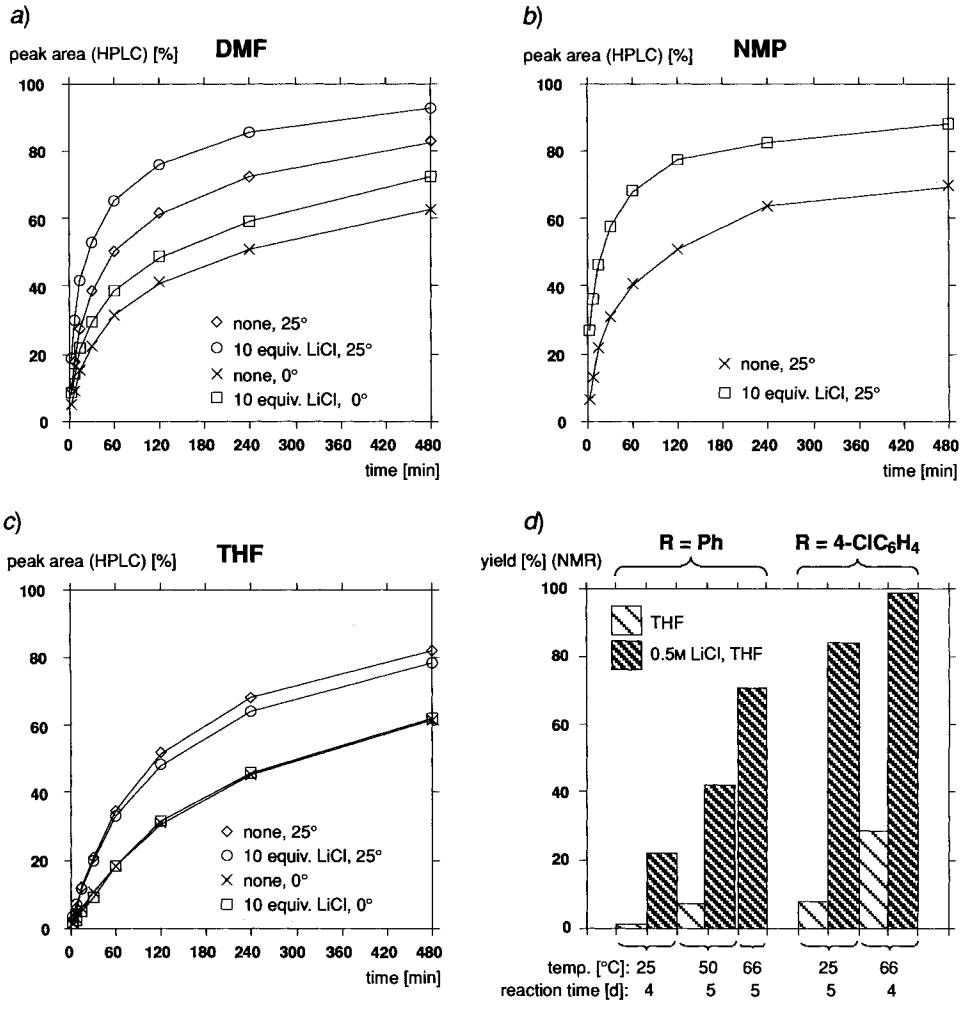

Figure. *Coupling of phenylalanine derivatives* (Z-Phe-OR) *with HCl·H-Ala-O(*t*-Bu)* (**11**) *using three different solvents and three different 'active' esters. a)–c)* Kinetics of the coupling of Z-Phe-ONp (**13**) with **11** in different solvents (*a*): DMF; *b*): NMP; *c*): THF). *d*): Coupling yields using different phenylalanine derivatives in THF/LiCl and varying the reaction temperature and time (no detailed description of the experiments which led to the construction of diagram *d*) are given in the *Exper. Part;* it was not checked whether epimerisation or side-product formation occurred in these experiments).

and LiCl were used. *N*-Acylurea **7** was not isolated, but this side product [28] may well have been formed to some extent in our reactions.

As a model for stepwise peptide synthesis and with a view to the use of Li-salts in solid-phase peptide synthesis, we also tested a urethane protecting group, *i.e.*, the Z protecting group, in our model coupling reactions. Urethane-type protecting groups on the N(α)-atom are known to protect amino-acid residues against epimerisation during activation and coupling [29]. Using Z-protection, no salt effects on the course of coupling

reactions using *symmetrical anhydrides* or *active esters* were observed. Both of these methods can, therefore, be recommended for stepwise coupling.

Coupling using the BOP [26] or TBTU [27] reagent gave somewhat more racemisation when Li-salts were present. Use of $LiBF_4$ appears to minimize this undesired effect.

Our results generally show that the use of $LiBF_4$ instead of LiCl gives less racemisation during couplings. Chloride ions formed during neutralisation of amino-acid hydrochlorides by Et_3N are known to enhance racemisation during coupling reactions due to their basicity and increase in ionic strength of the solvent [30]. One the other hand, *Lewis* acids such as $SnCl_4$, $TiCl_4$, $SbCl_3$, and $AlCl_3$ are known to lower racemisation [31]. Copper and zinc halides have been used to suppress racemisation in DCCI- and DCCI/HOBt-mediated coupling reactions in DMF [32]. Again, as in our experiments with DCCI and Li-salts, only low yields are obtained with copper and zinc halides, unless HOBt is present in the reaction mixture [32]. Our results are qualitatively in agreement with findings in solid-phase peptide synthesis [10] which show that LiCl decelerates DCCI couplings but does not affect symmetrical-anhydride couplings.

We conclude that both, anions and cations of salt additives, can affect peptide-coupling reactions. An anion effect is seen in our kinetic experiments where LiCl accelerated an active-ester coupling (*Fig.*), whereas $LiBF_4$ or $LiClO_4$ were without effect. A negative cation effect is postulated for $ZnCl_2$ which almost stopped the coupling reaction[11]).

As shown here, Li-salts greatly affect product distribution, racemisation, and kinetics of peptide-coupling reactions. We have identified reaction conditions which should allow for the safe use of Li-salts. These may be applied to peptide syntheses in order to overcome certain problems with the limited solubility of intermediates and products. For a study of Li-salts in solid-phase peptide synthesis, see the accompanying paper [21].

We thank Miss *U. Zweifel* and Mr. *Ch. Beerli* (Preclinical Research, *Sandoz Pharma AG*, Basel) for expert technical help.

Experimental Part

General. Inorganic salts were dried at 180° under high vacuum (h.v.) and stored in a desiccator over P_2O_5. Medium-pressure column chromatography: silica gel *60* (40–63 μm, *Merck*) using AcOEt/hexane 4:6 to 100% AcOEt. TLC: silica gel *60* F_{254} (*Merck*), detection with Cl_2/TDM (*N,N,N′,N′*-tetramethyl-4,4′-methylenebis[aniline]) reagent [33]. HPLC: *LiChrosorb 60 RP-8 Select B* (10 μm, 4.5 × 250 mm, *Merck*) using Me_4NOH buffers *A* and *B* (*A*: 900 ml of H_2O, 100 ml of MeCN, 2 ml of H_3PO_4 (85%), and 20 ml of Me_4NOH (10%, *Merck*); *B*: 300 ml of H_2O, 700 ml of MeCN, 2 ml of H_3PO_4 (85%), and 20 ml of Me_4NOH (10%, *Merck*)); UV detection at 205 nm. ^{1}H-NMR: *Varian Gemini 200* (200 MHz); $CDCl_3$; δ in ppm relative to internal Me_4Si, *J* in Hz, and integrals (*I*) relative to each other.

Procedure 1: The reaction mixture was added to 200 ml of AcOEt (150 ml of AcOEt in a second separatory funnel), the extract washed successively with 1N HCl (100 ml), 1N HCl (50 ml), 1M $KHCO_3$ (100 ml), 1M $KHCO_3$ (50 ml), and H_2O (2 × 50 ml), dried ($MgSO_4$), and evaporated, and the residue dried for several h under reduced pressure.

Procedure 2: The reaction mixture was worked up as in *Procedure 1*, the residue dissolved in $CHCl_3$ (80 ml), dicyclohexylurea (DCU) filtered off, and the filtrate chromatographed (column 3 × 40 cm, silica gel, 3–4 bar): DCU was eluted with AcOEt/hexane 4:6 and the product with AcOEt[12]).

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[11]) Effect observed during kinetic measurements (curves not shown in this paper).

[12]) It was checked, whether any enrichment of one of the diastereoisomers occurred during chromatography. This was not the case.

1. *Preparation of Dipeptides.* Reference compounds used for the *Halpern-Weinstein* test were prepared using standard procedures [34].

Z-Phe-Ala-O (t-*Bu*): ^{1}H-NMR: 7.15–7.40 (*m*, 10 arom. H); 6.33 (*d*, J = 5, NH); 5.29 (*d*, J = 5, NH); 5.10 (*s*, $CH_2(Z)$); 4.33–4.50 (*m*, H–C(2.1)); 4.35 (*quint.*, J = 6, H–C(2.2)); 3.13 (*dd*, ^3J = 6, ^2J = 14, 1 H–C(3.1)); 3.03 (*dd*, ^3J = 7, ^2J = 15, 1 H–C(3.1)); 1.45 (*s*, *t*-Bu); 1.31 (*d*, J = 6, CH_3(3.2)).

Z-D-*Phe-Ala-O*(t-*Bu*): ^{1}H-NMR: 7.15–7.42 (*m*, 10 arom. H); 6.18 (*d*, J = 6, NH); 5.39 (*d*, J = 6, NH); 5.10 (*s*, PhCH_2OCO); 4.30–4.50 (*m*, H–C(2.1)); 4.38 (*quint.*, J = 6, H–C(2.2)); 3.12 (*dd*, ^3J = 6, ^2J = 14, 1 H–C(3.1)); 3.02 (*dd*, ^3J = 8, ^2J = 14, 1 –H–C(3.1)); 1.42 (*s*, *t*-Bu); 1.19 (*d*, J = 6, CH_3(3.2)).

Ac-Phe-Ala-OMe: ^{1}H-NMR: 7.17–7.36 (*m*, 5 arom. H); 6.37 (*d*, J = 5, NH); 6.22 (*d*, J = 5, NH); 4.68 (*td*, J = 8, 6, H–C(2.1)); 4.47 (*quint.*, J = 7, H–C(2.2)); 3.71 (*s*, MeO); 3.11 (*dd*, ^3J = 6, ^2J = 12, 1 H–C(3.1)); 3.02 (*dd*, ^3J = 8, ^2J = 14, 1 H–C(3.1)); 1.98 (*s*, Ac); 1.34 (*d*, J = 6, CH_3(3.2)).

Ac-D-*Phe-Ala-OMe*: ^{1}H-NMR: 7.19–7.38 (*m*, 5 arom. H); 6.18 (*d*, J = 5, NH); 6.10 (*d*, J = 5, NH); 4.67 (*td*, J = 8, H–C(2.1)); 4.46 (*quint.*, J = 6, H–C(2.2)); 3.71 (*s*, MeO); 3.13 (*dd*, ^3J = 6, ^2J = 14, 1 H–C(3.1)); 2.96 (*dd*, J = 9, ^2J = 14, 1 H–C(3.1)); 2.00 (*s*, Ac); 1.19 (*d*, J = 6, CH_3(3.2)).

2. *Coupling Experiments* (*Tables 1–4*). 2.1. *Ac-Phe-Ala-OMe* (**3**). 2.1.1. *Mixed Anhydride, THF, NMM, –20°*; a) *no Salt*, b) *10 equiv. of LiCl*, c) *10 equiv. of $LiClO_4$*, *or* d) *10 equiv. of $LiBF_4$*. A soln. of **1** (207 mg, 1 mmol) and NMM (0.12 ml, 1.1 mmol) in THF (10 ml) was cooled to –20° and treated under stirring with isobutyl chloroformate (0.13 ml, 1 mmol). After 5 min, a cool soln. of **2** (140 mg, 1 mmol) and *a*) no salt, *b*) LiCl (424 mg, 10 mmol), *c*) $LiClO_4$ (1.064 g, 10 mmol), or *d*) $LiBF_4$ (937 mg, 10 mmol) in THF (10 ml), neutralized with NMM (0.12 ml, 1.1 mmol), was added. After stirring for 21 h and allowing to reach r.t., the mixture was worked up according to *Procedure 1*.

a) 219 mg (75%) of **3**. ^{1}H-NMR: 3.77 (*s*, MeO (**4**), I = 2.7); 3.71 (*s*, MeO (**3**), I = 40.2); **3**/**4** 94:6; < 3% D,L-isomer.

b) 224 mg (77%) of **3**. ^{1}H-NMR: 3.77 (*s*, MeO (**4**), I = 24.0); 3.71 (*s*, MeO (**3**), I = 37.2); **3**/**4** 61:39; < 3% D,L-isomer.

c) 238 mg (81%) of **3**. ^{1}H-NMR: 3.77 (*s*, MeO (**4**), I = 6.2); 3.71 (*s*, MeO (**3**); I = 14.6); no signals at *ca.* 1.19; **3**/**4** 70:30; < 3% D,L-isomer.

d) 185 mg (63%) of **3**. ^{1}H-NMR: 3.77 (*s*, MeO (**4**), I = 5.8); 3.71 (*s*, MeO (**3**), I = 48.2); no signals at *ca.* 1.19; **3**/**4** 89:11; < 3% D,L-isomer.

2.1.2. *Mixed Anhydride, THF, Et_3N, r.t.*; a) *no Salt*, b) *0.5 M LiCl, or* c) *0.5 M $LiClO_4$*. A soln. of **1** (207 mg, 1 mmol), Et_3N (0.14 ml, 1 mmol), and *a*) no salt, *b*) LiCl (212 mg, 5 mmol), or *c*) $LiClO_4$ (532 mg, 5 mmol) in THF (5 ml) was treated under stirring with isobutyl chloroformate (0.13 ml, 1 mmol) at r.t. After 5 min, a cool soln. of **2** (140 mg, 1 mmol) in THF (5 ml), neutralized with Et_3N (0.14 ml, 1 mmol), was added. The mixture was worked up according to *Procedure 1* after stirring for 16 h.

a) 130 mg (44%) of **3**. ^{1}H-NMR: 1.35, (*d*, J = 6, CH_3(3.2), L,L, I = 23.4); 1.22 (*d*, J = 6, CH_3(3.2), D,L, I = 41.5); only trace of **4**; 64% of D,L-isomer.

b) 130 mg (44%) of **3**. ^{1}H-NMR: 1.35 (*d*, J = 6, CH_3(3.2), L,L, I = 19.2); 1.22 (*d*, J = 6, CH_3(3.2), D,L, I = 7.6); 0.93 (*d*, J = 6, $(CH_3)_2CHCH_2$, **4**, I = 92.5); **3**/**4** 37:63; 28% D,L-isomer.

c) 190 mg (65%) of **3**. ^{1}H-NMR: 1.35 (*d*, J = 6, CH_3(3.2), L,L, I = 43.0); 1.22 (*d*, J = 6, CH_3(3.2), D,L, I = 17.8); 0.93 (*d*, J = 6, $(CH_3)_2CHCH_2$, **4**, I = 52.2); **3**/**4** 70:30; 29% D,L-isomer.

2.1.3. *Mixed Anhydride, THF, Et_3N, r.t.*; 5 *equiv. of LiCl*. A soln. of **1** (207 mg, 1 mmol) and Et_3N (0.14 ml, 1 mmol) in THF (5 ml) was treated under stirring with isobutyl chloroformate (0.13 ml, 1 mmol). After 5 min, a cool soln. of **2** (140 mg, 1 mmol) and LiCl (212 mg, 5 mmol) in THF (5 ml), neutralized with Et_3N (0.14 ml, 1 mmol), was added. After stirring for 6.5 h, the mixture was worked up according to *Procedure 1* leading to 228 mg (78%) of **3**. ^{1}H-NMR: 1.42 (*d*, J = 6, CH_3 (Ala of **4**), I = 14.5); 1.35 (*d*, J = 6, CH_3(3.2), L,L, I = 18.9); 1.22 (*d*, J = 6, CH_3(3.2), D,L, I = 15.2), **3**/**4** 70:30; 45% D,L-isomer.

2.1.4. *DCCI, THF, r.t.*; a) *no Salt*, b) *0.5 M LiCl, or* c) *0.5 M $LiClO_4$*. A suspension of **2** (140 mg, 1 mmol) and NMM (0.2 ml, 1.8 mmol) in THF (20 ml) was added to a soln. of **1** (207 mg, 1 mmol) and *a*) no salt, *b*) LiCl (848 mg, 20 mmol), or *c*) $LiClO_4$ (2.128 g, 20 mmol) in THF (20 ml). After addition of DCCI (206 mg, 1 mmol) at r.t. and stirring for 6 h, the mixture was worked up according to *Procedure 2*.

a) 192 mg (66%) of **3**. ^{1}H-NMR: 1.35 (*d*, J = 6, CH_3(3.2), L,L, I = 33.6); 1.22 (*d*, J = 6, CH_3(3.2), D,L, I = 11.0); 25% D,L-isomer.

b) 59 mg of an oily residue, which contained only traces of **3** as judged from the ^{1}H-NMR.

c) 72 mg (25%) of the slightly impure **3**. ^{1}H-NMR: 1.35 (*d*, J = 6, CH_3(3.2), L,L, I = 14.7); 1.20 (*d*, J = 6, CH_3(3.2), D,L, I = 8.2); 36% D,L-isomer.

2.1.5. *DCCI, DMF, r.t.;* a) *no Salt* b) *0.5* M *LiCl, or 0.5* M *$LiClO_4$*. A suspension of **2** (140 mg, 1 mmol) and NMM (0.2 ml, 1.8 mmol) in DMF (20 ml) was added to a soln. of **1** (207 mg, 1 mmol) and *a*) no salt, *b*) LiCl (848 mg, 20 mmol), or *c*) $LiClO_4$ (2.128 g, 20 mmol) in DMF (20 ml). After addition of DCCI (206 mg, 1 mmol) at r.t. and stirring for 3 h, the mixture was worked up according to *Procedure 2*.

a) 120 mg (41%) of **3**. ^{1}H-NMR: 1.35 (*d*, $J = 6$, CH_3(3.2), L,L, $I = 26.8$); 1.22 (*d*, $J = 6$, CH_3(3.2), D,L, $I = 16.0$); 37% D,L-isomer.

b) 62 mg (21%) of **3**. ^{1}H-NMR: 1.35 (*d*, $J = 6$, CH_3(3.2), L,L, $I = 45.0$); 1.22 (*d*, $J = 6$, CH_3(3.2), D,L, $I = 20.5$); 31% D,L-isomer.

c) 70 mg (24%) of **3**. ^{1}H-NMR: 1.35 (*d*, $J = 6$, CH_3(3.2), L,L, $I = 43$); 1.20 (*d*, $J = 6$, CH_3(3.2), D,L, $I = 24$); 36% D,L-isomer.

2.1.6. *DCCI/HOBt, THF, −20°→r.t.;* a) *no Salt or* b) *0.5* M *LiCl*. A suspension of **2** (140 mg, 1 mmol) and NMM (0.15 ml, 1.4 mmol) in THF (20 ml) was added at −20° to a soln. of **1** (207 mg, 1 mmol) and *a*) no salt or *b*) LiCl (848 mg, 20 mmol) in THF (20 ml). After addition of HOBT (135 mg, 1 mmol) and DCCI (206 mg, 1 mmol), the mixture was stirred for 48 h allowing to reach r.t. and worked up according to *Procedure 2*.

a) 259 mg (89%) of **3**. ^{1}H-NMR: 1.35 (*d*, $J = 6$, CH_3(3.2), L,L); no signal at 1.22; < 3% D,L-isomer.

b) 80 mg (27%) of **3**. ^{1}H-NMR: 1.35 (*d*, $J = 6$, CH_3(3.2), L,L, $I = 57.0$); 1.22 (*d*, $J = 6$, CH_3(3.2), D,L, $I = 5.9$); 9% D,L-isomer.

2.1.7. *DCCI/HOBt, DMF, −22°→r.t;* a) *no Salt,* b) *6 equiv. of LiCl, or* c) *6 equiv. of $LiBF_4$*. A suspension of **2** (140 mg, 1 mmol) and NMM (0.15 ml, 1.4 mmol) in DMF (20 ml) was added at −22° to a soln. of **1** (207 mg, 1 mmol) and *a*) no salt, *b*) LiCl (254 mg, 6 mmol), or *c*) $LiBF_4$ (562 mg, 6 mmol) in DMF (20 ml). After addition of HOBt (270 mg, 2 mmol) and DCCI (206 mg, 1 mmol), the mixture was stirred for 20 h allowing to reach r.t. and worked up according to *Procedure 2*.

a) 182 mg (62%) of **3**. ^{1}H-NMR: 1.35 (*d*, $J = 6$, CH_3(3.2), L,L, $I = 54$); 1.22 (*d*, $J = 6$, CH_3(3.2), D,L, $I = 2$); 4% D,L-isomer.

b) 191 mg (65%) of **3**. ^{1}H-NMR: 1.35 (*d*, $J = 6$, CH_3(3.2), L,L, $I = 56$); 1.21 (*d*, $J = 6$, CH_3(3.2), D,L, $I = 7.5$); 12% D,L-isomer.

c) 210 mg (72%) of **3**. ^{1}H-NMR: 1.35 (*d*, $J = 6$, CH_3(3.2), L,L, $I = 60.2$); 1.22 (*d*, $J = 6$, CH_3(3.2), D,L, $I = 2.7$); 4% D,L-isomer.

2.1.8. *TBTU Reagent According to [27];* a) *no Salt,* b) *6 equiv. of LiCl, or* c) *6 equiv. of $LiBF_4$*. A soln. of **1** (207 mg, 1 mmol), **2** (145 mg, 1.04 mmol), Et_3N (0.28 ml, 2 mmol), and *a*) no salt, *b*) LiCl (254 mg, 6 mmol), or *c*) $LiBF_4$ (562 mg, 6 mmol) in DMF (15 ml) at r.t. was treated with TBTU reagent **9** (334 mg, 1.04 mmol) stirred for 2 h, and worked up according to *Procedure 1*.

a) 251 mg (86%) of **3**. ^{1}H-NMR: 1.35 (*d*, $J = 6$, CH_3(3.2), L,L, $I = 56$); 1.22 (*d*, $J = 6$, CH_3(3.2), D,L, $I = 7.3$); 12% D,L-isomer.

b) 246 mg (84%) of **3**. ^{1}H-NMR: 1.35 (*d*, $J = 6$, CH_3(3.2), L,L, $I = 46.9$); 1.21 (*d*, $J = 6$, CH_3(3.2), D,L, $I = 14.0$); 23% D,L-isomer.

c) 237 mg (81%) of **3**. ^{1}H-NMR: 1.35 (*d*, $J = 6$, CH_3(3.2), L,L, $I = 53.5$); 1.22 (*d*, $J = 6$, CH_3(3.2), D,L, $I = 10.0$); 16% D,L-isomer.

2.1.9. *BOP Reagent According to [26];* a) *no Salt,* b) *6 equiv. of LiCl,* c) *or 6 equiv. of $LiBF_4$*. A soln. of **1** (207 mg, 1 mmol) **2** (145 mg, 1.04 mmol), Et_3N (0.28 ml, 2 mmol), and *a*) no salt, *b*) LiCl (254 mg, 6 mmol) or *c*) $LiBF_4$ (562 mg, 6 mmol) in DMF (15 ml) was treated at r.t., after 3 min stirring, with BOP reagent **8** (460 mg, 1.04 mmol). The mixture was stirred for 3.5 h and worked up according to *Procedure 1*.

a) 271 mg (93%) of slightly impure **3**. ^{1}H-NMR: 1.35 (*d*, $J = 6$, CH_3(3.2), L,L, $I = 50$); 1.22 (*d*, $J = 6$, CH_3(3.2), D,L, $I = 7.2$); 13% D,L-isomer.

b) 268 mg (92%) of slightly impure **3**. ^{1}H-NMR: 1.35 (*d*, $J = 6$, CH_3(3.2), L,L, $I = 43.5$); 1.21 (*d*, $J = 6$, CH_3(3.2), D,L, $I = 16.0$); 27% D,L-isomer.

c) 269 mg (92%) of slightly impure **3**. ^{1}H-NMR: 1.35 (*d*, $J = 6$, CH_3(3.2), L,L, $I = 46$); 1.22 (*d*, $J = 6$, CH_3(3.2), D,L, $I = 9.8$); 18% D,L-isomer.

2.2. *(Z-Phe)$_2$O* (**10**). A soln. of Z-Phe-OH (5.72 g, 19.1 mmol) in MeCN (50 ml) was cooled to −5° and treated with DCCI (1.97 g, 9.55 mmol). After stirring for 15 h and allowing to reach r.t., the mixture was separated from the formed urea by filtration, the filtrate evaporated, and the white residue crystallized from MeCN: 4.566 g (82%) of **10**. M.p. 138° ([35]: 128–129°).

2.3. *Z-Phe-Ala-O(*t*-Bu)* (**12**). 2.3.1. *Symmetrical Anhydride;* a) *no Salt or* b) *5 equiv. of LiCl*. A soln. of **11** (91 mg, 0.5 mmol), Et_3N (0.074 ml, 0.52 mmol), and *a*) no salt or *b*) LiCl (106 mg, 2.5 mmol) in DMF (7 ml) was cooled to −20°. After 5 min, a cooled soln. of **10** (290 mg, 0.5 mmol) in DMF (15 ml) was added. After allowing to reach r.t. and stirring for 4 days, the mixture was worked up according to *Procedure 2*.

a) 200 mg (94%) of **12**. ^{1}H-NMR: 1.30 (*d*, *J* = 6, CH_3(3.2), L,L); no signal at 1.20; < 3% D,L-isomer.

b) 201 mg (94%) of **12**. ^{1}H-NMR: 1.30 (*d*, *J* = 6, CH_3(3.2), L,L); no signal at 1.20; < 3% D,L-isomer.

2.3.2. *4-Nitrophenyl Ester, THF;* a) *no Salt or* b) *6 equiv. of LiCl.* After neutralizing a soln. of **13** (420 mg, 1 mmol), **11** (182 mg, 1 mmol), and a) no salt or b) LiCl (254 mg, 6 mmol) in THF (30 ml) with NMM (0.11 ml, 1 mmol), the mixture was stirred for 24 h at r.t. and worked up according to *Procedure 1*.

a) 377 mg (88%) of **12**. ^{1}H-NMR: 1.30 (*d*, *J* = 6, CH_3(3.2), L,L); no signal at 1.20 ppm); < 3% D,L-isomer.

b) 389 mg (91%) of **12**. ^{1}H-NMR: 1.30 (*d*, *J* = 6, CH_3(3.2), L,L); no signal at 1.20 ppm); < 3% D,L-isomer.

2.3.3. *4-Nitrophenyl Ester, DMF;* a) *no Salt or* b) *6.4 equiv. of LiCl.* After neutralizing a soln. of **13** (210 mg, 0.5 mmol), **11** (91 mg, 0.5 mmol), and *a*) no salt or *b*) LiCl (137 mg, 3.2 mmol) in DMF (20 ml) at −10° with NMM (0.11 ml, 1 mmol), the mixture was stirred for 3.5 h allowing to reach r.t. and worked up according to *Procedure 1*.

a) 197 mg of a mixture of 39% of **12** and 61% of **13** (based on ^{1}H-NMR). ^{1}H-NMR: 3.25 (*d*, *J* = 6, 2 H–C(3.1(**13**)), *I* = 11; 3.08 (*d*, *J* = 6, 2 H–C(3.1(**12**)), *I* = 7; 1.30 (*d*, *J* = 6, CH_3(3.2), L,L); no signal at 1.20; < 3% D,L-isomer.

b) 198 mg of a mixture of 44% of **12** and 56% of **13** (based on ^{1}H-NMR). ^{1}H-NMR: 3.25 (*d*, *J* = 6, 2 H–C(3.1(**13**)), *I* = 10; 3.08 (*d*, *J* = 6, 2 H–C(3.1(**12**)), *I* = 8; 1.30 (*d*, *J* = 6, CH_3(3.2),L,L); no signal at 1.20; < 3% D,L-isomer.

2.3.4. *Pentachlorophenyl Ester;* a) *no Salt,* b) *6 equiv. of LiCl,* or *c*) *6 equiv. of $LiBF_4$.* A soln. of **14** (548 mg, 1 mmol) in DMF (10 ml) was cooled to −40° and treated under stirring with a non-cooled soln. of **11** (182 mg, 1 mmol) and *a*) no salt, *b*) LiCl (254 mg, 6 mmol), or *c*) $LiBF_4$ (562 mg, 6 mmol) in DMF (10 ml), neutralized with NMM (0.145 ml, 1.3 mmol). After stirring for 18 h and allowing to reach r.t., the mixture was worked up according to *Procedure 1*.

a) 372 mg (87%) of **12**. ^{1}H-NMR: 1.30 (*d*, *J* = 6, CH_3(3.2), L,L); no signal at 1.20; < 3% D,L-isomer.

b) 392 mg (92%) of **12**. ^{1}H-NMR: 1.30 (*d*, *J* = 6, CH_3(3.2), L,L); no signal at 1.20; < 3% D,L-isomer.

c) 381 mg (89%) of **12**. ^{1}H-NMR: 1.30 (*d*, *J* = 6, CH_3(3.2), L,L); no signal at 1.20; < 3% D,L-isomer.

3. *Kinetic Experiments for Active-Ester Coupling. Z-Phe-Ala-O(*t*-Bu)* (**12**). To a soln. of **11** (45 mg, 0.25 mmol) in 4 ml of solvent (containing no additive or 2.5 mmol of salt) were added NMM (55 µl, 0.5 mmol) and **13** (105 mg, 0.25 mmol) in 2 ml of solvent. After 3, 7, 15, 30, 60, 120, 240 and 480 min samples (40 µl) were removed and added to Et_2O (2 ml) and 1N HCl (1 ml). The org. layer was separated and evaporated and the residue dissolved in MeOH (1 ml) and analysed by HPLC (100% B). The peak area of **12** is given in % of the total peak area of **12** and **13** (measured by integration of the UV (205 nm) signals). Conditions: *a*) DMF, 0°: 3 (4.9), 7 (9.1), 15 (15.2), 30 (22.4), 60 (31.4), 120 (41.0), 240 (50.8), and 480 min (62.7). *b*) DMF, LiCl, 0°: 3 (8.8), 7 (14.0), 15 (21.6), 30 (29.5), 60 (38.7), 120 (48.7), 240 (59.1), and 480 min (72.3). *c*) DMF, r.t.: 3 (9.7), 7 (17.9), 15 (27.3), 30 (38.5), 60 (50.3), 120 (61.4), 240 (72.4), and 480 min (82.9). *d*) DMF, LiCl, r.t.: 3 (19.0), 7 (30.1), 15 (41.9), 30 (53.2), 60 (65.2), 120 (76.0), 240 (85.7), and 480 min (93.0). *e*) THF, 0°: 3 (1.5), 7 (3.1), 15 (5.9), 30 (10.6), 60 (18.4), 120 (30.8), 240 (45.5), and 480 min (61.5). *f*) THF, LiCl, 0°: 3 (1.4), 7 (2.6), 15 (5.1), 30 (9.2), 60 (18.3), 120 (31.6), 240 (46.0), and 480 min (62.0). *g*) THF, r.t.: 3 (2.7), 7 (5.8), 15 (12.0), 30 (20.9), 60 (34.8), 120 (51.7), 240 (68.5), and 480 min (82.2). *h*) THF, LiCl, r.t.: 3 (3.4), 7 (6.9), 15 (11.8), 30 (20.0), 60 (33.1), 120 (48.2), 240 (64.1), and 480 min (78.7). *i*) NMP, r.t.: 3 (6.6), 7 (13.2), 15 (21.8), 30 (30.9), 60 (40.5), 120 (49.2), 240 (63.6), and 480 min (69.8). *k*) NMP, LiCl, r.t.: 3 (26.8), 7 (36.1), 15 (46.6), 20 (57.4), 60 (68.1), 120 (77.7), 240 (82.8), and 480 min (88.2).

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