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Synthesis of symmetric and asymmetric diamides of citric acid and amino acids

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Summary. A convenient method for the synthesis of symmetric and asymmetric diamides of amino acids including DOPA and citric acid from 2-*tert*-butyl-1,3-di(N-hydroxysuccinimidyl)citrate and 1-*tert*-butyl-2,3-di(N-hydroxysuccinimidyl)citrate is described.

Keywords: Amino acids – Citric acid amides – Synthesis – DOPA – Amino acid derivatives

Abbreviations: AcO*t*Bu: *tert*-butyl acetate, i-Bu: iso-butyl, *t*Bu: *tert*-butyl, Bzl: benzyl, p-OH-Bzl: p-hydroxybenzyl, m,p-$(OH)_2$-Bzl: m,p-dihydroxybenzyl, DCCI: dicyclohexylcarbodiimide, Et: ethyl, Me: methyl, Su: succinimidyl, SuOH: N-hydroxysuccinimide, Ph: phenyl

Introduction

Low molecular weight compounds, able to form stable complexes with various metal ions in a selective manner are the subject of growing interest in different areas of chemistry including analytical chemistry, inorganic chemistry, medicinal chemistry. Although the structural requirements of a particular compound to be effective as a chelating agent depend on the metal ion to be complexed, two characteristic features are common: presence of specific functional groups acting as donates complexing the metal ion and the proper carbon backbone, size and shape of which must be precisely optimized in order to minimize any possible steric repulsions decreasing the stability of the complex formed with a particular metal ion.

Citric acid can serve as an ideal backbone donating compound for the construction of chelating agents. Citric acid itself is known as a calcium chelating agent under physiological conditions (Milewska, 1988; Goodwin, 1968). Moreover, the presence of one hydroxyl and three carboxyl groups offers an opportu-

nity of introducing "spacer arms" containing required functional groups in order to construct more effective chelating agents. Nature has already taken an advantage of citric acid in the family of citric acid – derived, specific ferric ion carriers – siderophores. This group consists of a few compounds including aerobactin, arthrobactin and schizokinen (Neilands, 1973; Chimiak, 1984; Bergeron, 1984; Hider, 1984).

It is worth mentioning that β-citryl-L-glutamic acid had been isolated from newborn rat brains (Miyake et al., 1978), although a physiological role of this compound is still unknown.

In this paper, we propose a simple and convenient method for the synthesis of asymmetric and symmetric diamides of citric acid and amino acids from 1-*tert*-butyl-2,3-di(N-hydroxysuccinimidyl)citrate (**5**) and 2-*tert*-butyl-1,3-di(N-hydroxysuccinimidyl)citrate (**6**), respectively. This method should be especially useful for the construction of synthetic chelators.

Materials and methods

Homogeneity of the products (**7**) and (**8**) were checked by TLC on silica gel 60 (Merck) with the following eluents: a) benzene – ethyl acetate – ethanol (7 : 2 : 1), b) *n*-butanol – acetic acid – water (4 : 1 : 1).

The esters (**1**) and (**2**) were obtained from citric acid (Hirota et al., 1980). *tert*-Butyl esters of amino acids were obtained by the method of Taschner (Taschner et al., 1961).

1-tert-Butyl-2,3-dimethyl citrate (**3**)

A mixture of 18 g (75 mmol) 1,2-dimethyl citrate (**2**) (Hirota et al., 1980), 150 ml (1113 mmol) *tert*-butyl acetate and 2.15 ml (26 mmol) $HClO_4$ left for 3 days at room temperature. Afterwards, the mixture was neutralized with a saturated $NaHCO_3$ solution and subsequently extracted with ether. Ether and the excess of *tert*-butyl acetate were removed under reduced pressure. After recrystallization (ether/hexane), the ester (**3**) (11.8 g, 59%); mp. 52–54°C was obtained.

$C_{12}H_{20}O_4$ (276,28)	calc.	C 52.17	H 7.30
	found	52.19	7.26

^{1}H-nmr (CCl_4/TMS): δ = 1.35 (s, 9H, tC_4H_9); 2.70 (s, 2H, CH_2CO); 2.75 (s, 2H, CH_2CO); 3.65 (s, 3H, CH_3); 3.80 (s, 3H, CH_3); 4.15 (s, 1H, OH).

1-tert-Butyl citrate (**4**)

27.6 g (0.1 mol) 1-*tert*-butyl-2,3-dimethyl citrate (**3**) in 100 ml methanol were hydrolyzed for 3 h with 100 ml 2N NaOH. Methanol was removed under reduced pressure; aqueous solution was acidified with 2N HCl to pH 3–4 and then extracted with ethyl acetate. Product (**4**) (17.3 g, 70%) was recrystallized from ether/hexane (mp. 118–119°C).

$C_{10}H_{16}O_7$ (248.33)	calc.	C 48.49	H 6.50
	found	48.45	6.52

^{1}H-nmr ($CDCl_3$/TMS): δ = 1.4 (s, 9H, tC_4H_9); 2.7 (d, 2H, CH_2CO); 2.85 (s, 2H, CH_2COOH).

1-tert-Butyl-2,3-di(N-hydroxysuccinimidyl) citrate (**5**)

To a solution of 2.48 g (10 mmol) 1-*tert*-butyl citrate (**4**) and 2.3 g (20 mmol) N-hydroxysuccinimide in 80 ml ethyl acetate, 4.52 g (22 mmol) dicyclohexylcarbodiimide in 10 ml ethyl acetate were added and the reaction mixture was stirred for 15 h at room temperature and subsequently left at 4°C for 2 h. After usual work up and recrystallization (ethyl acetate/ether) the ester (**5**) (3.5 g; 80%) mp. 146–149°C was obtained.

$C_{18}H_{22}O_{11}N_2$ (442.38)	calc.	C 48.87	H 5.01	N 6.33
	found	48.35	4.98	6.43

^{1}H-nmr ($CDCl_3$/TMS): δ = 1.4 (s, 9H, tC_4H_9); 2.8 (s, 10H, $(CH_2)_2$, CH_2CO); 3.0 (s, 2H, CH_2CO); 4.7 (s, 1H, OH).

3,4-Dihydroxy-L-phenylalanine tert-butyl ester p-toluenesulfonate

The solution containing 5 g (25 mmol) L-DOPA and 2.26 ml (27.5 mmol) $HClO_4$ in 225 ml (1670 mmol) *tert*-butyl acetate was vigorously stirred for 4–5 days at the room temperature. The reaction mixture was worked up according to the procedure described by Taschner (Taschner et al., 1961). After recrystallization (ethyl acetate/ether) the ester (4.46 g, 42%), mp. 133–136°C, $[\alpha]_D^{20}$-6° (c = 2, MeOH), TLC: R_f (4 : 1 : 1/nBuOH : H_2O : AcOH) 0.52, was obtained.

$C_{20}H_{27}O_7NS$ (425.48)	calc.	C 56.45	H 6.40	N 3.29
	found	56.57	6.60	2.95

^{1}H-nmr (D_2O): δ = 1.3 (s, 9H, tC_4H_9); 2.3 (s, 3H, CH_3); 3.05 (d, 2H, CH_2); 4.1 (t, 1H, CH); 6.7 (m, 3H, C_6H_3); 7.65 (d-d, 4H, C_6H_4)
ir: $\nu_{C=O}$ 1740 cm^{-1}

General procedure for compounds (**7**)

To a mixture of 2.5 mmol of the active ester of *tert*-butyl citrate (**5**) or (**6**) and 5.5 mmol of the salt of the amino acid esters in 50 ml dioxane, 5.5 mmol of triethylamine or N-methylmorpholine were added dropwise and stirred for few hours. Afterwards the solvent was removed under reduced pressure and the oily residue was dissolved in chloroform. The resulting solution was washed with water, 5% aqueous citric acid solution, water, saturated $NaHCO_3$ solution, water and dried over $MgSO_4$. After crystallization amides (**7**) were obtained.

Particularly, compound (**7i**) was purified by column chromatography on silica gel (Merck, 70–270 mesh), eluent: hexane – *i*-propanol (5 : 1 v/v).

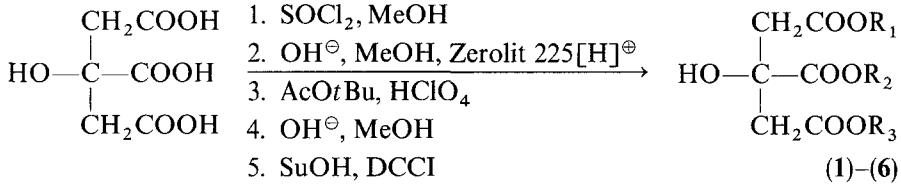
General procedure for compounds (**8**)

2.5 mmol of compound (**7**) were treated with 5 ml (60 mmole) trifluoroacetic acid for few hours. The solvent was removed under reduced pressure and the residue (**8**) was crystallyzed.

Results and discussion

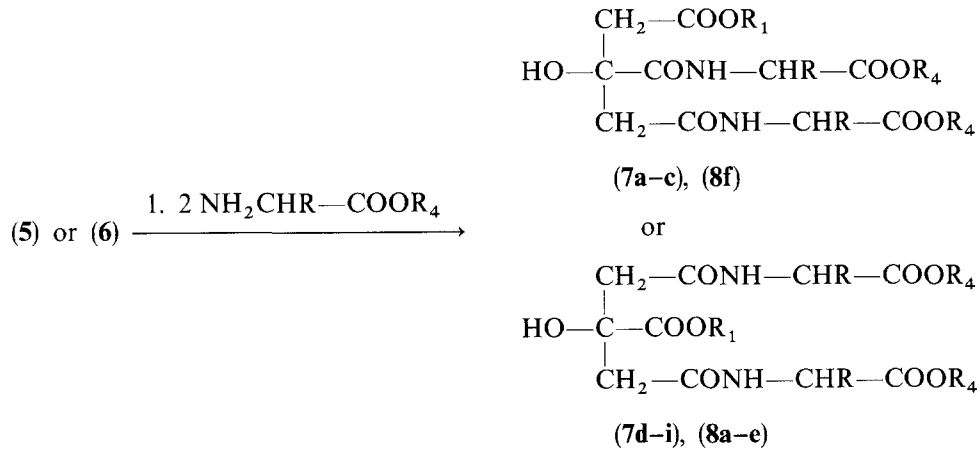
Citric acid was first converted into trimethyl citrate (**1**) by the esterification with methanol in the presence of thionyl chloride. Citrate (**1**) was partially hydrolyzed to 1,2-dimethyl citrate (**2**), which was subsequently transesterified with *tert*-butyl acetate in the presence of perchloric acid. Citrate (**3**) was again subjected to alkaline hydrolysis. Reaction of the 1-*tert*-butyl citrate (**4**) with N-hydroxy-

succinimide in the presence of DCCI afforded 1-*tert*-butyl-2,3-di(N-hydroxysuccinimidyl)citrate (**5**) with a good yield. Citrate (**6**) was described previously (Milewska, 1987).



	(1)	(2)	(3)	(4)	(5)	(6)
R_1	Me	H	*t*Bu	*t*Bu	*t*Bu	Su
R_2	Me	Me	Me	H	Su	*t*Bu
R_3	Me	Me	Me	H	Su	Su

Finally citrate (**5**) and (**6**) were converted into the respective asymmetric and symmetric diamides (**7**) in the reaction with amino acid esters. The *tert*-butyl groups could be selectively removed with trifluoroacetic acid.



	R	R_4	R_1
7a	H	Et	*t*Bu
7b	Bzl	*t*Bu	*t*Bu
7c	p-HO-Bzl	*t*Bu	*t*Bu
7d	H	Et	*t*Bu
7e	Me	*t*Bu	*t*Bu
7f	*i*Bu	Me	*t*Bu
7g	Bzl	*t*Bu	*t*Bu
7h	p-HO-Bzl	*t*Bu	*t*Bu
7i	m, p-$(HO)_2$-Bzl	*t*Bu	*t*Bu
8a	H	Et	H
8b	*i*Bu	Me	H
8c	Bzl	H	H
8d	p-HO-Bzl	H	H
8e	m, p-$(HO)_2$-Bzl	H	H
8f	Bzl	H	H

The physical constants and spectroscopic data for all the final compounds (**7**) and (**8**) are collected in Tables 1 and 2.

Table 1. Physical data of compounds (**7**) and (**8**)

No compd.	Reaction time [h]	Yield [%]	m.p. [°C] solvent	$[\alpha]_D^{20}$	R_f TLC
7a	15	64	81–82 ether/hexane	—	0.35[a]
7b	18	75	109–110 ether/hexane	+51° c = 1.9 $CHCl_3$	0.7[a]
7c	24	79	78–80 ether/hexane	+35° c = 1.3 $CHCl_3$	0.4[a]
7d	10	80	94–95 $CHCl_3$/hexane	—	0.37[a]
7e	12	87	53–55 ether/hexane	−6° c = 2.4 $CHCl_3$	0.58[a]
7f	15	80	99–101 ether/hexane	−1.5° c = 4 $CHCl_3$	0.57[a]
7g	15	69	112–113 ether/hexane	+23.6° c = 5 $CHCl_3$	0.7[a]
7h	48	71	168–171 $CHCl_3$/hexane	+10° c = 4 EtOH	0.5[a]
7i	24	65	144–146 ether/hexane	+27° c = 2 $CHCl_3$	0.33[a]
8a	3	87	98–99 acetone/ether	—	0.46[b]
8b	2	90	135–137 ethyl acetate/hexane	low	0.7[b]
8c	2	89	161–163 acetone/ether	+20° c = 3.5 acetone	0.5[b]
8d	2.5	92	102–108 acetone/ether	+18° c = 1.2 acetone	0.55[b]
8e	3	90	168–170 acetone/ether	n.d.	0.25[b]
8f	2	80	amorphous	n.d.	0.7[b]

[a] benzene-ethyl acetate-ethanol (7:2:1)
[b] *m*-butamol-acetic acid-water (4:1:1)

Table 2. The microanalysis and spectral data of compounds (**7**) and (**8**)

No	Mol. formula (m. weight)	Microanalysis CHN [%] calcd.	found	irC [cm^{-1}] KBr	^1H NMR (solvent) [ppm]
7a	$C_{18}H_{30}N_2O_9$	51.67	51.87	1770	CCl_4: δ = 1.2 (t, 6H, CH_3);
		7.23	7.36	1750	1.4 (s, 9H, tC_4H_9); 2.5 (d, 2H,
	(418.3)	6.69	6.76	1690	CH_2CO); 2.7 (s, 2H, CH_2CO);
				1550	6.0 (s, 1H, OH); 7.8 (m, 2H, NH)
7b	$C_{36}H_{50}N_2O_9$	66.03	65.89	1760	CCl_4: δ = 1.4 (s, 27H, tC_4H_9);
		7.70	7.88	1740	2.5 (m, 4H, CH_2); 3.0 (m, 4H, CH_2);
	(654.8)	4.28	4.08	1680	4.6 (t, 2H, CH); 5.9 (d, 1H, OH);
				1530	7.1 (s, 10H, C_6H_5); 7.3–8.0 (m,
					2H, NH)
7c	$C_{36}H_{50}N_2O_{11}$	62.95	63.12	1760	$CDCl_3$: δ = 1.5 (s, 27H, tC_4H_9);
		7.34	7.33	1740	2.6 (m, 4H, CH_2); 3.05 (m, 4H, CH_2)
	(686.8)	4.08	4.22	1660	4.7 (m, 2H, CH); 6.5–7.3 (m, 8H,
				1540	C_6H_4)
7d	$C_{16}H_{30}N_2O_9$	51.67	51.81	1740	$CDCl_3$: δ = 1.2 (t, 6H, CH_3); 1.4
		7.23	7.35	1640	(s, 9H, tC_4H_9); 2.7 (s, 4H, CH_2CO)
	(418.3)	6.69	6.69	1530	4.0 (d, 4H, CH_2CO); 4.2 (q, 4H,
					CH_2); 5.0 (s, 1H, OH); 7.3 (t, 2H, NH)
7e	$C_{24}H_{42}N_2O_9$	57.35	57.12	1730	CCl_4: δ = 1.3 (d, 6H, CH_3); 1.4
		8.42	8.58	1640	(s, 27H, tC_4H_9); 2.55 (m, 4H,
	(502.4)	5.57	5.47	1520	CH_2CO); 4.3 (qv, 2H, CH), 5.0 (m,
					1H, OH); 7.5 (t, 2H, NH)
7f	$C_{24}H_{42}N_2O_9$	57.35	57.60	1730	CCl_4: δ = 0.9 (d, 12H, $(CH_3)_2$);
		8.42	8.60	1640	1.4 (s, 9H, tC_4H_9); 1.6 (t, 4H, CH_2)
	(502.4)	5.57	5.41	1520	1.3–1.8 (m, 2H, CH); 2.6 (s, 4H,
					CH_2, CHN); 5.1 (s, 1H, OH), 7.55
					(t, 2H, NH)
7g	$C_{36}H_{50}N_2O_9$	66.03	65.86	1720	$CDCl_3$: δ = 1.37 (s, 27H, tC_4H_9);
		7.70	7.83	1650	2.55 (d, 4H, CH_2CO); 3.0 (d, 4H,
	(654.8)	4.28	4.21	1520	CH_2), 4.45 (q, 2H, CH); 5.0 (s,
					1H, OH), 7.23 (s, 12H, C_6H_5, NH)
7h	$C_{36}H_{50}N_2O_{11}$	62.95	63.01	1740	$CDCl_3$: δ = 1.4 (s, 27H, tC_4H_9);
		7.34	7.39	1650	2.5 (d, 4H, CH_2CO); 2.95 (m, 4H,
	(686.8)	4.08	3.89	1510	CH_2Ph); 4.95 (s, 1H, OH); 6.8
					(m, 8H, C_6H_4)
7i	$C_{36}H_{50}N_2O_{13}$	60.15	60.45	1720	$CDCl_3$: δ = 1.35 (s, 27H, tC_4H_9);
		7.07	7.14	1640	2.53 (m, 4H, CH_2CO); 2.9 (m, 4H,
	(718.5)[a]	3.90	3.86	1515	CH_2); 4.7 (m, 3H, CH, OH); 6.63
					(m, 6H, C_6H_3); 7.1 (t, 2H, NH)
8a	$C_{14}H_{22}N_2O_9$	46.41	46.38	1740	D_2O: δ = 1.2 (t, 6H, CH_3); 2.9 (s,
		6.12	6.25	1730	4H, CH_2CO); 4.0 (s, 4H, CH_2); 4.2
	(362.3)	7.73	7.76	1640	(q, 4H, CH_2CH_3)
				1530	

Table 2 (cont.)

No	Mol. formula (m. weight)	Microanalysis ir CHN [%] calcd.	found	[cm^{-1}] KBr	^1H NMR (solvent) [ppm]
8b	$C_{20}H_{34}N_2O_9$	53.99	60.12	1750	TFA: δ = 0.8 (d, 12H, CH_3); 1.5
		8.06	7.91	1730	(t, 4H, CH_2); 1.3–1.8 (m, 2H, CH);
	(446.3)	6.27	6.41	1640	2.8 (s, 4H, CH_2CO); 3.5 (s, 6H,
				1540	$COOCH_3$); 4.35 (q, 2H, CHN); 7, 6 (d, 2H, NH)
8c	$C_{24}H_{26}N_2O_9$	59.25	59.29	1730	D_2O: δ = 2.55 (d, 4H, CH_2CO); 3.1
		5.39	5.25	1720	(d, 4H, CH_2Ph); 4.6 (m, 2H, CH);
	(486.5)	5.76	5.95	1640	7.3 (s, 10H, C_6H_5)
				1530	
8d	$C_{24}H_{26}N_2O_{11}$	55.60	55.71	1730	D_2O: δ = 2.57 (m, 4H, CH_2CO); 2.9
		5.05	5.14	1720	(m, 4H, CH_2); 4.57 (m, 2H, CH_2N);
	(518.5)	5.40	5.26	1650	6.67, 6.8, 6.97, 7.1 (dd, 8H, C_6H_4)
				1515	
8e	$C_{24}H_{26}N_2O_{13}$	52.36	52.56	1730	D_2O: δ = 2.55 (m, 4H, CH_2CO);
		4.76	4.78	1720	2.95 (m, 4H, CH_2Ph); 4.5 (m, 2H,
	(550.5)	5.09	5.21	1650	CH); 6.7 (m, 6H, C_6H_3)
				1520	
8f	$C_{24}H_{26}N_2O_9$	59.25	59.31	1720	
		5.39	5.23	1710	
	(486.5)	5.76	5.85	1660	
				1640	
				1530	

[a] MS (FD, 18 mA): 719.5 (M + 1)

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