(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 3 524 596 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
09.09.2020 Bulletin 2020/37

(51) Int Cl.:
C07D 251/46 *(2006.01)*

(21) Application number: **19165931.7**

(22) Date of filing: **22.12.2015**

(54) **REAGENTS COMPRISING 2-HALO-4,6-DIALKOXY-1,3,5-TRIAZINES IN THE PRESENCE OF AMINES AND THEIR USE IN A STABILIZATION METHOD.**

REAGENZIEN MIT 2-HALO-4,6-DIALKOXY-1,3,5-TRIAZINEN IN ANWESENHEIT VON AMINEN SOWIE DEREN VERWENDUNG IN EINEM STABILISIERUNGSVERFAHREN

RÉACTIFS COMPRENANT 2-HALO-4,6-DIALKOXY-1,3,5-TRIAZINES EN PRÉSENCE D'AMINES AINSI QUE SON APPLICATION DANS UN PROCÉDÉ DE STABILISATIONN

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR

(30) Priority: **23.12.2014 IT VE20140070**
23.12.2014 IT VE20140071

(43) Date of publication of application:
14.08.2019 Bulletin 2019/33

(62) Document number(s) of the earlier application(s) in accordance with Art. 76 EPC:
15830862.7 / 3 237 390

(73) Proprietor: **Crossing S.r.l.**
31100 Treviso (TV) (IT)

(72) Inventor: **BEGHETTO, Valentina**
I-30172 Mestre (VE) (IT)

(74) Representative: **Sarpi, Maurizio et al**
Studio Ferrario S.r.l.
Via Collina, 36
00187 Roma (IT)

(56) References cited:
US-A- 4 076 538 **US-A1- 2013 219 634**
US-B2- 8 679 196

- **BOWES J H ET AL: "Crosslinking of Collagen", JOURNAL OF APPLIED CHEMISTRY, WILEY, US, vol. 15, no. 7, 1 July 1965 (1965-07-01), pages 296-304, XP002750889, ISSN: 0021-8871, DOI: 10.1002/JCTB.5010150702 [retrieved on 2007-05-04]**

EP 3 524 596 B1

Description

Field of application of the invention

[0001] The present invention relates to the sector of activating agents for condensation, crosslinking, grafting, and curing reactions that intervene in the processes of stabilization of collagen matrices, and for the condensation of natural and synthetic polymers.

[0002] In particular, the invention regards 2-halo-4,6-dialkoxy-1,3,5-triazines, which act as activating agents for condensation, crosslinking, grafting, and curing reactions, and for stabilization of collagen matrices, as well as for the condensation of polymers, and the multiple applications of said reagents in various industrial sectors, amongst which the tanning industry and the leather-processing industry.

Prior art

[0003] Commonly, amides, esters, and thioesters are formed from the reaction between an amine, alcohol, thioalcohol, and an "activated" carboxylic acid, i.e., obtained by formation of acyl chlorides, mixed anhydrides, or activated esters. These reactions underlie processes for production of a vast range of products in the most disparate sectors, such as those of pharmaceuticals, polymers, packaging, foodstuffs, tissues, etc.

[0004] In particular, carbodiimides are organic reagents widely used for the formation of amide bonds, ester bonds, thioester bonds, etc., in so far as they are able to react with carboxylic acids to form an active intermediate species, which, in the presence of an amine, alcohol, or thioalcohol, reacts to form the desired bond [A. El-Faham, Chem. Rev., 2011, 111, 6557-6602]. One of the carbodiimides most frequently used is dicyclohexylcarbodiimide (DCC); however, during the reaction, DCC leads to the formation of a toxic coproduct that must be carefully removed at the end of the reaction. Reactions in the presence of carbodiimides are prevalently carried out in organic solvent, since these molecules are not stable in aqueous solution, except for 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide chlorohydrate (EDC). EDC, however, calls for the combined use of equimolar amounts (or higher amounts) of N-hydroxysuccinimide (NHS), which is rather unstable and must be stored at low temperature (approximately -20°C) and is very expensive. Currently, this reagent is in any case one of the most widely used for the production of polyaminoacids and of other pharmaceutical derivatives with high added value, as well as for crosslinking of collagen, for the reconstruction of tendons and retinas, the production of hydrogels, etc. [US 8,691,279, US 2012/0009223 A1].

[0005] In the biotechnology sector, carbodiimides (for example, EDC/NHS) are widely used as alternatives to glutaraldehyde for crosslinking of collagen thanks to their lower toxicity. However, the properties of materials crosslinked with the exclusive use of carbodiimides, the gelatinization temperature (Tg), and the physico-mechanical properties are clearly inferior.

[0006] To obtain collagen matrices having characteristics comparable to those obtained with glutaraldehyde, acyl azides, and glycerol [E. Khor, Biomaterials, 1997, 18, 95-105], carbodiimides are normally used in the presence of crosslinking agents that remain permanently attached to the collagen tissue [X. Duan, Biomaterials, 2006, 27, 4608-4615].

[0007] It is known that the derivatives of 2-halo-4,6-dialkoxy-1,3,5-triazines, and in particular their quaternary ammonium salts, represent a valid alternative to carbodiimides and can be used, also in an aqueous environment, for the formation of amide bonds, ester bonds, and thioester bonds by means of reactions of crosslinking, grafting, curing, etc. in homogeneous and/or heterogeneous phase [US 6,458,948 B1, Z.J. Kaminski, J. Am. Chem. Soc., 2005, 127, 16912-16920]. In a large number of cases, these reagents are more efficient than other coupling agents known to date, such as DCC, EDC/NHS, PyBOP, HATU, HBTU, etc. An alternative, at present rarely employed, is to resort to the use of the quaternary ammonium salts of 2-halo-4,6-dialkoxy-1,3,5-triazines, and in particular 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) (the only one commercially available), for stabilization of complex matrices for medical use, made up of collagen in combination with other natural and/or synthetic matrices [EP1748985 B1, US 2008/0234254 A1, US 2011/118265 A1, US 8,119,592, WO 2010/056778A].

[0008] The quaternary ammonium salts of 2-halo-4,6-dialkoxy-1,3,5-triazines do not present problems of toxicity in the end products since they are not withheld therein and can be easily eliminated at the end of the treatment/reaction. For these reasons, the scientific literature regarding DMTMM has undergone continuous growth in the last few years. For instance, the international patent application No. WO 2014/063102 presents the use of DMTMM for the preparation of artificial lubricants for cartilage. The derivatives of 2-halo-4,6-dialkoxy-1,3,5-triazines are, however, very sensitive to the solvent, and this constitutes a limit to their use. Up to the present day, the literature regarding the synthesis of derivatives of 2-halo-4,6-dialkoxy-1,3,5-triazines is rather limited and in all cases involves at least two steps: 1) synthesis of the triazine derivative from the corresponding 2-halo-4,6-dialkoxy-1,3,5-triazine in the presence of an amine in a given solvent, normally an organic one; 2) recovery and purification of the product before use [US 6,458,948B1; US 2003/0153785A1; EU174962B1/2006; WO2007/051496A1; S. S.A. Raw, Tetrah. Lett., 2009, 50, 946-948]. However, this protocol, which is generally used for the synthesis of organic compounds, also referred to as "Isolated-Product

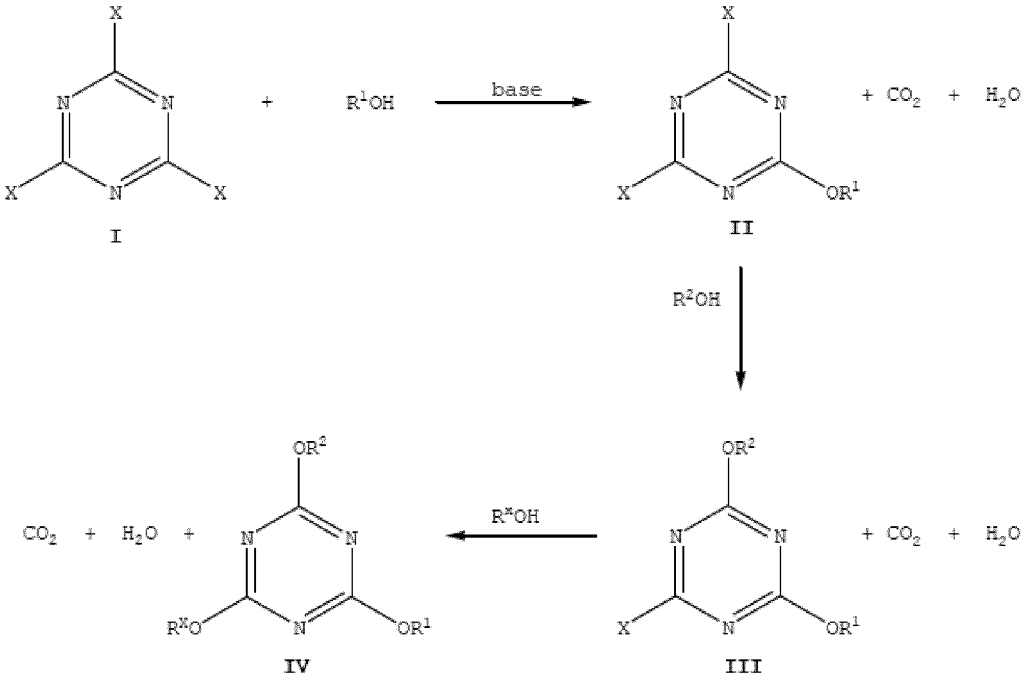
Protocol" (IPP), presents a certain number of critical features, above all from the standpoint of industrial production, in so far as it calls for complex reactors, large amounts of solvents, complicated purification steps, etc., which moreover reduce considerably the yield in the desired product, leading to an increase in the operating costs and hence sales prices.

[0009] M. Kunishima *et al.* have studied the mechanism of reaction of dehydrocondensations in the presence of quaternary ammonium salts of 2-halo-4,6-dialkoxy-1,3,5-triazines [Chem. Eur. J. 2012, 18, 15856-15867]. The authors give some examples of reactions conducted in CH_2Cl_2, a solvent in which the quaternary ammonium salts of 2-halo-4,6-dialkoxy-1,3,5-triazines are highly unstable, leading to rapid decomposition. To overcome this problem, the authors present some examples of reactions between a carboxylic acid and a primary amine, in the presence of CDMT and a tertiary amine, but probably on account of the solvent used (CH_2Cl_2) and the absence of buffers, auxiliaries, etc., in the majority of cases the main product obtained is the product of condensation between the triazine and the primary amine, instead of the desired amide. Currently, only 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM), are commercially available, at very high prices, on account of the lack of an adequate process on an industrial scale for their production (in terms of kilograms per day or tonnes per day) .

[0010] In the recent literature, there have been described many examples of application that use DMTMM obtained by means of IPP, which, however, have some trouble in finding a use at an industrial level also on account of the problems linked to the use of DMTMM (high costs, low availability, instability over time, etc.) [US 2013/0123508 A1, EP 1992364 A1]. DMTMM has a cost that is over three hundred times the average cost of equivalent activating agents currently used for the synthesis of polymers, biomaterials, and leather. Furthermore, quaternary ammonium salts of 2-halo-4,6-dialkoxy-1,3,5-triazines are generally unstable at room temperature over long periods [US 2003/0153785 A1] and may be subject to partial or total decomposition if they are not shipped and stored in adequate conditions. To garantee conservation thereof, DMTMM must be shipped and stored at -20°C. The cost of DMTMM is directly linked to the cost and availability of CDMT from which it is synthesised.

[0011] The literature regarding synthesis of 2-halo-4,6-dialkoxy-1,3,5-triazines principally regards the preparation of CDMT. One of the fundamental aspects of the synthesis of CDMT, as likewise of 2-halo-4,6-dialkoxy-1,3,5-triazines in general, is the control of the course of the reaction in order to minimize or eliminate completely formation of secondary products.

[0012] Currently, the only protocol for the synthesis of 2-halo-4,6-dialkoxy-1,3,5-triazines is described in US 2002/0123628 and regards the synthesis on a scale of some grams carried out with normal laboratory equipment. The reaction is generally conducted starting from a cyanuric chloride in the presence of an alcohol, prevalently methanol, and a base, preferably $NaHCO_3$. During the reaction, water and CO_2 are formed. In the document No. US 2002/0123628, the authors pose as basic requisite for obtaining good selectivity and yields of 2-halo-4,6-dialkoxy-1,3,5-triazines that the amount of water present at the start and at the end of the reaction should always be less than 2.5 mol per mole of cyanuric halogenide (compound of formula I, hereinafter also referred to simply as "I", in the reaction sheme presented below). Consequently, since water is a byproduct of the reaction, to obtain high yields of CDMT according to the protocol described above, it is necessary for all the solvents to be distilled and anhydrified prior to use and possibly for the reactions to be conducted in an inert atmosphere. Furthermore, large amount of alcohol is required, used both as reagent and as solvent for reducing the viscosity of the system (ratio alcohol/I = 5-50 mol/mol). At the end of the reaction, the product must be recovered by extraction with water/organic solvents and then anhydrified, and the organic solvent evaporated. Presented in the scheme appearing below is the synthesis of 2-halo-4,6-dialkoxy-1,3,5-triazines, together with the secondary products that may form during the reaction.



where:

R^x is R^1 or R^2; R^1 and R^2 are chosen independently from: $-CH_3$, $-CH_2CH_3$, $-CH(CH_3)_2$, $-(CH_2)_2CH_3$, $-(CH_2)_3CH_3$; and X is Br^- or Cl^-.

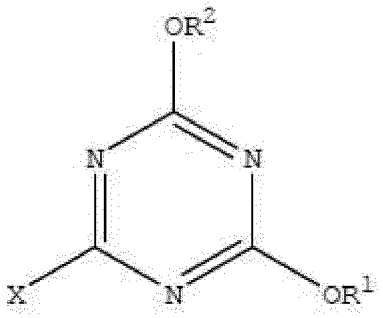
[0013] According to Dudley [J. Am. Chem. Soc., 1951, 73, 2986-2990] the addition of variable amounts of water for the synthesis of CDMT improves the homogeneity of the system (ratio I/base/H_2O/MeOH=1/2/2/8). However, the author does not analyse the evolution of the kinetics of formation of the compound of formula IV as the rate of addition of the reagents varies. On the basis of our studies it has been found that, if the addition of the compound of formula I is too fast (exothermic) variable percentages of the compound of formula IV are formed (from 5 to 25%), with consequent higher consumption of methanol. Consequently, this procedure calls for large amounts of methanol, with yields of less than 74%. J. Cronin [Synth. Commun., 1996, 26, 3491-3494] in his work presents a methodology employed exclusively for the synthesis of CDMT, which, according to the author, can be used for up to a maximum of 20 kg. However, no detail as regards scale up is presented, and in effect, we have been unable to reproduce Cronin's protocol for amounts exceeding 50 g of CDMT, and complex mixtures containing compounds of formulas II, III, and IV were produced in variable percentages.

Summary of the invention

[0014] A purpose of the invention is to provide 2-halo-4,6-dialkoxy-1,3,5-triazines and compositions that include them as agents for activating reactions of condensation, crosslinking, grafting, and curing. These reagents are used in processes for tanning hides, where their application is particularly advantageous. In this particular form of reaction, the invention envisages that stabilization of the hide is obtained by means of reaction with two reagents, in succession or previously mixed, one of which comprises one or more 2-halo-4,6-dialkoxy-1,3,5-triazines, and the other comprises one or more linear, branched, aromatic, cyclic, heterocyclic alkyl tertiary amines.

Detailed description of the invention

[0015] It has been found that compounds of formula III,



(III)

where:
R^1 and R^2 are independently chosen from: $-CH_3$, $-CH_2CH_3$, $-CH(CH_3)_2$, $-(CH_2)_2CH_3$, $-(CH_2)_3CH_3$; and X is Cl^- or Br^-
namely, 2-halo-4,6-dialkoxy-1,3,5-triazines, are able to act as agents for activating reactions of condensation, crosslinking, grafting, and curing and in processes of stabilization of collagen matrices, as well as of condensation of natural and synthetic polymers, such as cellulose, and/or modified celluloses, polysaccharides, starch, lignin, etc., and their application is very advantageous in terms of ease of use, economic convenience, and stability over time of these compounds.

[0016] Thus, through a specific method that uses them, which forms the subject of the invention described herein, it is possible to reduce the overall costs considerably as compared to the methods used for the same purpose in the prior art and reduce the environmental impact of the process, limiting the amount of solvents, energy, and time necessary for their preparation and implementation.

[0017] The method of stabilization of collagen matrices and of condensation of natural and synthetic polymers that forms the subject of the present invention hence presents as a methodology alternative to preparation using IPP.

[0018] In particular, the method of stabilization of collagen matrices and of condensation of polymers that forms the subject of the present invention is obtained from the reaction of two reagents, denoted, for the purposes of the present invention, as "first reagent", or "Reagent 1", and "second reagent", or "Reagent 2".

[0019] According to the present invention, Reagent 1 is a composition comprising:

a) at least one compound of formula III (2-halo-4,6-dialkoxy-1,3,5-triazine);
b) a buffer;
c) a salt;
d) a solvent.

[0020] According to the present invention, Reagent 2 is a composition comprising:

a) a tertiary amine;
b) a buffer;
c) a solvent.

[0021] Reagent 2 may further comprise an additive for the buffer.

[0022] Hence, forming the subject of the present invention are also the compositions of the aforesaid two reagents, Reagent 1 and Reagent 2, which are essential for implementation of the method according to the invention.

[0023] Reagent 1 is a composition comprising as active principle one or more 2-halo-4,6-dialkoxy-1,3,5-triazines in a concentration ranging between 0.1M and 1.0M. The composition that constitutes Reagent 1 also comprises a buffer, preferably a Good buffer, chosen in the group: MES, ACES, BES, BIS-Tris, MOPS, TEA, TAPSO, POPSO, TAPS, formiate, phosphate, succinate. The composition that constitutes Reagent 1 comprises a base or a salt of formula X^+Y^-, where X^+ is Na^+, K^+, or Ag^+, and Y^- is : ClO_4^-, BF_4^-, PF_6^-, CO_3^{2-}, Cl^-, HCO_3^-.

[0024] The composition that constitutes Reagent 1 comprises a solvent chosen in the group of: aliphatic ethers, halogenates, alcohols, ketones, esters, aromatic or aliphatic hydrocarbons, amides, carbonates, DMSO, and water.

[0025] Reagent 2 is a composition comprising as active principle one or more linear, branched, cyclic, aromatic, heterocyclic tertiary amines, and/or their quaternary salts, in a concentration ranging between 0.1M and 1.0M. The composition that constitutes Reagent 2 also comprises a buffer, preferably a Good buffer, chosen in the group: HEPES, MOPS, TRIS, tri-Na-citrate, Tris-Cl, TAPS.

[0026] The composition that constitutes Reagent 2 comprises a solvent chosen in the group of: aliphatic ether, halogenate, alcohol, ketone, ester, aromatic or aliphatic hydrocarbon, amide, carbonate, DMSO, and water.

[0027] In some particularly preferred embodiments, Reagent 2 may further comprise additives for the buffer, which are chosen in the group: NaCl, Na_2HPO_4, NaOAc, KCl, SDS, glycine, boric acid, EDTA, and NaN_3.

[0028] The process of stabilization of the collagen matrices according to the invention finds application in multiple contexts of considerable technological and industrial interest.

[0029] Up to the present day, there does not exist any protocol that uses 2-halo-4,6-dialkoxytriazines in the presence of amines for stabilization of collagen. The present applicant has conducted tests that demonstrate that the method forming the subject of the invention enables crosslinking of powdered collagen dispersed in water by adding one after the other the two reagents, Reagent 1 and Reagent 2, as described in the examples provided hereinafter. From the results of the experimentation, it emerges clearly that the procedure adopted for crosslinking the collagen according to the invention conducted in the presence of Reagents 1 and 2, is notably superior to the one obtained with IPP. In particular, Reagents 1 and 2 have proved to present a better performance than aldehydes, glycerol, synthetic/natural crosslinking agents, carbodiimides, EDC/NHS, and quaternary ammonium salts of 2-halo-4,6-dialkoxy-1,3,5-triazines, which are currently used for stabilization of collagens according to the prior art, where normally the gelatinization temperature (Tg) does not exceed 80°C.

[0030] The above result is particularly important for the production of collagen tissues and materials with high thermal stability and their conservation in time for medical use and use in biotechnology (collagen, leather, tissues, corneas, etc.).

Experimental part

[0031] The invention will be described in what follows by way of non-limiting illustration, with particular reference to some examples.

[0032] In the examples presented hereinafter for non-limiting illustrative purposes, Reagents 1 and 2 according to the invention are identified and represented with the codes AaBbCcDdEe and FfGgHhEe, where a, b, c, d, ... = 0, 1, 2, 3, 4, ... n.

[0033] In particular, for Reagent 1:

A identifies 2,4-dialkoxy-1,3,5-triazines; for example, A_1: 2-chloro-4,6-dimethoxy-1,3,5-triazine; A_2: 2-chloro-4,6-diethoxy-1,3,5-triazine; A_3: 2-chloro-4methyl-6-ethyl-1,3,5-triazine, etc.
B identifies the buffer, preferably a Good buffer; for example, B_1: MES; B_2: ACES; B_3: BES, B_4: POPSO; B_5: TRIS; B_6: HEPPSO; B_7: TAPS; B_8: Tris-NaCitrate.
C identifies the cation of an inorganic salt X^+; for example, C_1: Na^+; C_2: K^+; C_3: Ag^+.
D identifies the anion of an inorganic salt Y^-; for example, D_1: ClO_4^-; D_2: BF_4^-; D_3: Cl^-; etc.
E identifies the solvent; for example, E_1: aliphatic ether; E_2: alcohol; E_3: water; E_4: acetone; E_5: THF; etc.

[0034] For Reagent 2:

F identifies the aliphatic, linear, branched, cyclic, aromatic, heterocyclic, amine and/or its quaternary salts, for example, F_1: TMA (trimethylamine); F_2: TEA (triethylamine), F_3: DEMA (diethylmethylamine); F_4: NMM (N-methylmorpholine); F_5: NEM (N-ethylmorpholine); F_6: MPD (methylpyrrolidine); F_7: MP (methylpiperidine); etc.
G identifies the buffer, preferably a Good buffer; for example, G_1: BES; G_2: MOPS; G_3: TRIS; G_4: POPSO, G_5: TAPS; G_6: Tris-NaCitrate; etc.
H identifies the additives for the buffer; for example, H_1: NaCl; H_2: Na_2HPO_4; H_3: NaOAc; H_4: KCl; H_5: SDS; etc.
E identifies the solvent; for example, E_1: aliphatic ether; E_2: alcohol; E_3: water; E_4: acetone; E_5: THF; etc.

[0035] All the analyses presented herein were carried out with a gas chromatograph Agilent Technologies 6850, using a flame-ionization detector, equipped with an HP5 capillary column (5% methylphenylsilicone; conditions of analysis: 50°C for 4 min, then 20°C/min up to 250°C). The ^1H and ^{13}C NMR spectra were recorded with a spectrometer Bruker Avance 300 operating at 300.11 MHz for the proton spectrum and at 75.03 MHz for the carbon spectrum. The FT-IR spectra (KBr tablet) were obtained with a spectrophotometer Perkin Elmer "Spectrum One". The DSC analyses were determined with DSC Netzsch STA 409 PC, melting point Buchi 535. The enantiomeric excesses were measured by means of chiral HPLC using a CHIRALCEL OD-H (250 mm x 4.6 mm) with an Agilent 1100 HPLC equipped with a 254-nm UV detector.

Example 1. Condensation between benzoic acid and phenethylamine (Test 2 of Table 1) by means of a procedure that uses Reagent 1 ($A_1B_1C_2D_3E_3$) and Reagent 2 ($F_6G_2H_2E_3$)

[0036] In a flask provided with magnetic stirring there were dissolved 293.1 mg (2.4 mmol) of benzoic acid in 15 mL of methanol. To the solution there were added 300 μL (2.4 mmol) 2 of phenethylamine and 2.4 mL of Reagent 1. Finally, there were added 2.4 mL of Reagent 2. After 2h a sample was taken for monitoring conversion, which was found to be

60%; then, the solvent was evaporated using a rotary evaporator. The solid residue was dissolved in diethyl ether (30 mL), and subsequently washed with a saturated solution of Na_2CO_3, water, a 1N solution of HCl, and a saturated solution of NaCl, anhydrified with $MgSO_4$, and filtered. The solution was dried off to obtain the product in the form of a white solid (450.6 mg, 2 mmol, yield 83%).

^1H NMR ($CDCl_3$, 300 MHz, ppm) δ_H: 7,72-7.31 (m, 2H), 7.52- 7.23 (m, 8H), 6.26 (br s, 1H), 3.73 (m, 2H), 2.95 (t, 2H) ; ^{13}C NMR (75 MHz, $CDCl_3$, ppm) δ_C:. 167.43, 138.86, 134.60, 131.33, 128.75, 128.65, 128.43, 126.76, 126.52, 41.10, 35.67.

Formulation of Reagent 1 ($A_1B_1C_2D_3E_3$): 1.0M solution of 2-chloro-4,6-dimethoxy-1,3,5-triazine, and of 10 wt% of MES, 0.5 wt% of KCl, and water.

Formulation of Reagent 2 ($F_6G_2H_2E_3$): 1.0M solution of MPD, 0.5-0.8 wt% of MOPS, 0.5-1.5 wt% of Na_2HPO_4, and water.

Example 1 - comparative test. Reaction of condensation between benzoic acid and phenethylamine (Test 1 of Table 1) with DMT-MPD with IPP method

Synthesis of DMT-MPD

[0037] In a flask provided with magnetic stirring there were introduced 500 mg (2.85 mmol) of CDMT dissloved in 10 mL of THF to which there were added drop by drop 300 μL (2.85 mmol) of MPD. After 10 min a white precipitate was obtained, which was recovered by filtration. The NMR analyses, in various solvents, highlighted that the product was not stable in solution, and hence it was not possible to carry out coupling with this reagent.

Table 1. Coupling reaction of benzoic acid and phenethylamine in the presence of triazine quaternary ammonium salts obtained with IPP and with the method that uses Reagent 1 and Reagent 2

Test	Activator	t (h)	Conv. (%)[a]
1	DMT-MPD[b]	-	-
2	$A_1B_1C_2D_3E_3/F_6G_2H_2E_3$	1 h	60
3	DET-EM[b]	-	-
4	$A_2B_2C_2D_1E_1/F_5G_4H_1E_1$	24 h	60
5	DET-TMA[b]	-	-
6	$A_2B_2C_2D_1E_3/F_1G_4H_1E_3$	1 h	100
7	DMT-MP	2 h	81
8	$A_1B_3C_2D_1E_4/F_7G_4H_1E_4$	2 h	90
9	DET-TMA	2 h	98
10	$A_1B_1C_2D_2E_5/F_1G_4H_1E_5$	2 h	100
11	DET-MM[b]	-	-
12	$A_2B_2C_2D_1E_3/F_4G_3H_1E_3$	2 h	70
13	DMT-MM[c]	2 h	96
14	$A_1B_4C_2D_2E_5/F_4G_3H_1E_5$	2 h	100
15	DET-EM	24 h	76
16	$A_1B_0C_2D_2E_4/F_5G_4H_1E_4$	24 h	82

Conditions of reaction: benzoic acid (1 Eq), phenethylamine (1 Eq), condensing agent (1 Eq).
[a] Conversion calculated by means of GLC with 156 mg (1 mmol) of n-undecane as internal standard.
[b] It was not possible to isolate the quaternary ammonium salt according to IPP.
[c] Commercially available.

Example 2 - comparative test. Condensation between benzoic acid and phenethylamine (Test 7 of Table 1) by means of IPP

[0038] In a flask provided with magnetic stirring there were dissolved 293.1 mg (2.4 mmol) of benzoic acid in 15 mL

of methanol. To the solution there were added 300 μL (2.4 mmol) of phenethylamine and 692 mg (2.4 mmol) of DMT-MP obtained with IPP. After 2 h a sample was taken for monitoring conversion, which was found to be 81%; then, the solvent was evaporated using a rotary evaporator. The solid residue was dissolved in diethyl ether (30 mL), and subsequently washed with a saturated solution of Na_2CO_3, water, a 1N solution of HCl, and a saturated solution of NaCl and then anhydrified in $MgSO_4$, and filtered. The solution was dried off to obtain the product as white solid (405.5 mg, 1.8 mmol, yield 75%).
^{1}H NMR ($CDCl_3$, 300 MHz, ppm) δ_H: 7.72-7.31 (m, 2H), 7.52-7.23 (m, 8H), 6.26 (br s, 1H), 3.73 (m, 2H), 2.95 (t, 2H) ; ^{13}C NMR (75 MHz, $CDCl_3$, ppm) δ_C: 167.43, 138.86, 134.60, 131.33, 128.75, 128.65, 128.43, 126.76, 126.52, 41.10, 35.67.

Example 3. Production of chiral amides with the method that uses Reagent 1 ($A_2B_3C_1D_1E_3$) and Reagent 2 ($F_4G_3H_1E_3$)

[0039] In a flask provided with magnetic stirring there were dissolved 200 mg (0.51 mmol) of 2-methyl-3-p-anisyl propanoic acid in 15 mL of methanol. To the solution there were added 55 μL (0.6 mmol) of aniline, 0.5 mL of Reagent 1 and, finally, 0.5 mL of Reagent 2. After 24 h the solvent was evaporated using a rotary evaporator. The solid residue was dissolved in ethyl ether (30 mL), and subsequently washed with a saturated solution of Na_2CO_3, water, a 1N solution of HCl, and a saturated solution of NaCl, and then anhydrified with $MgSO_4$, and filtered. The solution was then dried off to obtain the product in the form of a yellow liquid with a yield of 75% (101 mg, 0.375 mmol).
^{1}H NMR ($CDCl_3$, 300 MHz, ppm) δ_H: 7.33-7.26 (m, 2H), 7.24-7.14 (m, 2H), 7.07-7.01 (m, 2H),7.01-6.95 (m, 1H), 6.75 (d, 2H), 3.69 (s, 3H), 2.95-2.80 (m, 1H), 2.70-2.56 (m, 1H), 2.55-2.35 (m, 1H), 1.19 (d, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz, ppm) δ_C: 172.95, 157.17, 136.68, 130.66, 128.89, 127.85, 123.18, 118.96, 112.91, 54.23, 44.02, 38.71, 16.67; HPLC: and.and. 96%, CHIRACEL OD-H column, n-hexane/isopropanol 92/8, 0.8 mL/min, t_R = 17.15 min (lower) and t_R =21.2 min (upper).
Formulation of Reagent 1 ($A_2B_3C_1D_1E_3$): 1.0M solution of 2-chloro-4,6-diethoxy-1,3,5-triazine, and of 0-6 wt% of BES, 0.5 wt% of $NaClO_4$, and water.
Formulation of Reagent 2 ($F_4G_3H_1E_3$): 0.5M solution of NMM, 0.1-0.8 wt% of Tris, 0.5-2.5 wt% of NaCl, and water.

Example 4. Funtionalization with aniline of polyacrylic acid with the method that uses Reagent 1 ($A_1B_4C_2D_1E_3$) and Reagent 2 ($F_4G_6H_2E_3$)

[0040] In a flask provided with magnetic stirring there were dissolved 60 mg (1.3×10^{-4} mmol) of PAA (MW = 450000) and 190 μL (2.1 mmol) of aniline in 35 mL of methanol. To the solution there were then added 2.1 mL of Reagent 1 and 2.1 mL of Reagent 2. The solution was left under stirring for 24 h and then the solid was filtered, washed, dried, and analysed by means of ^{1}H NMR.
Formulation of Reagent 1 ($A_1B_4C_2D_1E_3$): 0.7M solution of 2-chloro-4,6-dimethoxy-1,3,5-triazine, and of 0-6 wt% of POPSO, 0.5-1.0 wt% of $KClO_4$, and water.
Formulation of Reagent 2 ($F_4G_6H_2E_3$): 0.7M solution of NMM, 0.1-5 wt% of Tris NaCitrate, 0.7-2.3 wt% of Na_2HPO_4, and water.

Example 5. Crosslinking of CMC with the method that uses Reagent 1 ($A_1B_4C_2D_1E_3$) and Reagent 2 ($F_4G_6H_2E_3$)

[0041] In a flask provided with magnetic stirring there were dissolved 279 mg of CMC (carboxymethylcellulose with carboxylation degree of 0.7) in 25 mL of water. To the solution there were then added 3 mL of Reagent 1 and 3 mL of Reagent 2. The solution was left under stirring for 24 h, and then the aqueous phase was evaporated by means of a high-vacuum pump. The solid obtained was washed with water and characterized by means of FT-IR.
FT-IR: 3200, 1750-1735, 1602, 1020 cm^{-1}
Formulation of Reagent 1 ($A_1B_4C_2D_1E_3$): 0.5M solution of 2-chloro-4,6-dimethoxy-1,3,5-triazine, and of 0-6 wt% of POPSO, 0.5-1.0 wt% of $KClO_4$, and water.
Formulation of Reagent 2 ($F_4G_6H_2E_3$): 0.5M solution of NMM, and of 0.1-5 wt% of Tris NaCitrate, 0.7-2.3 wt% of Na_2HPO_4, and water.

Example 6. Funtionalization with methanol of polyacrylic acid with the method that uses Reagent 1 ($A_2B_4C_2D_1E_3$) and Reagent 2 ($F_4G_6H_2E_3$)

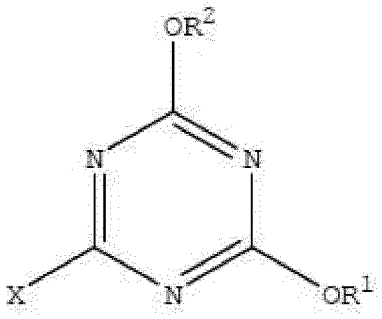
[0042] In a flask provided with magnetic stirring there were dissolved 1.65 g (3.8×10^{-2}mmol) of an aqueous solution at 35% of sodium salt of polyacrylic acid (PAANa, MW = 15000) and 2 mL of methanol in 60 mL of water. To the solution there were then added 5 mL of Reagent 1 and 5 mL of Reagent 2. The solution was left under stirring for 24 h, and washed with etyl ether. The aqueous phase was concentrated using a high-vacuum pump, and the solid obtained was

analysed by means of ^{1}H NMR.
^{1}H NMR (D_2O, 300 MHz, ppm) δ_H: 2.94 (s, 0.48H), 2.47 (br s, 1H), 1.66 (m, 2H).
Formulation of Reagent 1 ($A_2B_4C_2D_1E_3$) : 0.2M solution of 2-chloro-4,6-diethoxy-1,3,5-triazine, and of 0-6 wt% of POPSO, 0.5-1.0 wt% of $KClO_4$, and water.
Formulation of Reagent 2 ($F_4G_6H_2E_3$) : 0.2M solution of NMM, 0.1-5 wt% of Tris NaCitrate, 0.7-2.3 wt% of Na_2HPO_4, and water.

Claims

1. A pair of reagents for stabilization of collagen matrices and for condensation of polymers, constituted by a first reagent comprising:

   a) at least one compound of formula III (2-halo-4,6-dialkoxy-1,3,5-triazine)



(III),

   where: R^1 and R^2 are independently chosen from: $-CH_3$,$-CH_2CH_3$, $-CH(CH_3)_2$, $-(CH_2)_2CH_3$, and $-(CH_2)_3CH_3$; and X is Cl^- or Br^-;
   b) a buffer;
   c) an inorganic salt;
   d) a solvent, and

   a second reagent that is a composition comprising:

   a) a tertiary amine, and/or a quaternary salt thereof;
   b) a buffer;
   c) a solvent.

2. The pair of reagents according to Claim 1, wherein the first reagent comprises as active principle one or more 2-halo-4,6-dialkoxy-1,3,5-triazines of formula III in a concentration ranging between 0.1 and 1.0 M.

3. The pair of reagents according to Claims 1 and 2, wherein the first reagent comprises a buffer, chosen in the group of:MES, ACES, BES, BIS-Tris, MOPS, TEA, TAPSO, POPSO, TAPS, formiate, phosphate, succinate; and a base or a salt of formula X^+Y^-, where X^+ is chosen from Na^+, K^+, and Ag^+, and Y^- is chosen from: ClO_4^- , BF_4^- , PF_6^- , CO_3^- , Cl^-, and HCO_3^-.

4. The pair of reagents according to any one of Claims 1 to 3, wherein the solvent of Reagent 1 is chosen in the group of: aliphatic ether, halogenate, alcohol, ketone, ester, aromatic or aliphatic hydrocarbon, amide, carbonate, DMSO, and water.

5. The pair of reagents according to any one of Claims 1 to 4, wherein the second reagent comprises as active principle one or more linear, branched, cyclic, aromatic, heterocyclic tertiary amines, and/or a quaternary salt thereof, in a concentration ranging between 0.1 and 1.0 M.

6. The pair of reagents according to any one of Claims 1 to 5, wherein the second reagent comprises a buffer, chosen in the group of: HEPES, MOPS, TRIS, tri-Na-citrate, Tris-Cl, and TAPS.

7. The pair of reagents according to any one of Claims 1 to 6, wherein the solvent of Reagent 2 is chosen in the group of: aliphatic ether, halogenate, alcohol, ketone, ester, aromatic or aliphatic hydrocarbon, amide, carbonate, DMSO, and water.

8. The pair of reagents according to any one of Claims 1 to 7, wherein the second reagent may further comprise an additive for the buffer.

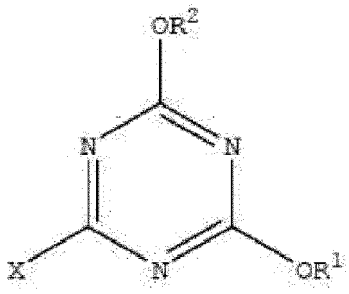
9. The pair of reagents according to Claim 8, wherein the additive for the buffer of the second reagent is chosen in the group of: NaCl, Na_2HPO_4, NaOAc, KCl, SDS, glycine, boric acid, EDTA, and NaN_3.

10. Use of the pair of reagents as to Claims 1 to 9 in a method for stabilization of collagen matrices and condensation of polymers by means of reactions of condensation, crosslinking, grafting, and curing.

Patentansprüche

1. Reagenzienpaar zur Stabilisierung von Kollagenmatrizen und zur Kondensation von Polymeren, bestehend aus einem ersten Reagenz, umfassend:

a) mindestens eine Verbindung gemäß Formel III (2-Halo-4,6-dialkoxy-1,3,5-triazin)



(III),

wobei: R^1 und R^2 unabhängig voneinander ausgewählt sind aus: $-CH_3$, $-CH_2CH_3$, $-CH(CH_3)$, $-(CH_2)_2CH_3$, und $-(CH_2)_3CH_3$; und X Cl^- oder Br^- ist;
b) einen Puffer;
c) ein anorganisches Salz;
d) ein Lösungsmittel, und

ein zweites Reagenz, welches eine Zusammensetzung ist, umfassend:

a) ein tertiäres Amin, und/oder ein quaternäres Salz davon;
b) einen Puffer,
c) ein Lösungsmittel.

2. Reagenzienpaar gemäß Anspruch 1, wobei das erste Reagenz ein oder mehrere 2-Halo-4,6-dialkoxy-1,3,5-triazine gemäß Formel III als Wirkprinzip in einer Konzentration zwischen 0.1 und 1.0 M umfasst.

3. Reagenzienpaar gemäß den Ansprüchen 1 und 2, wobei das erste Reagenz einen Puffer umfasst, ausgewählt aus der Gruppe bestehend aus: MES, ACES, BES, BIS-Tris, MOPS, TEA, TAPSO, POPSO, TAPS, Formiat, Phosphat, Succinat; und eine Base oder ein Salz der Formel X^+Y^-, wobei X^+ ausgewählt ist aus Na^+, K^+, und Ag^+, und Y^- ausgewählt ist aus: ClO_4^-, BF_4^-, PF_6^-, CO_3^-, Cl^-, und HCO_3^-.

4. Reagenzienpaar gemäß einem der Ansprüche 1 bis 3, wobei das Lösungsmittel des Reagenz 1 ausgewählt ist aus der Gruppe bestehend aus: aliphatische Ether, Halogene, Alkohole, Ketone, Ester, aromatische oder aliphatische Kohlenwasserstoffe, Amide, Carbonate, DMSO, und Wasser.

5. Reagenzienpaar gemäß einem der Ansprüche 1 bis 4, wobei das zweite Reagenz ein oder mehrere lineare, verzweigte, cyclische, aromatische, heterocyclische tertiäre Amine, und/oder quaternäre Salze davon als Wirkprinzip

in einer Konzentration zwischen 0.1 und 1.0 M umfasst.

6. Reagenzienpaar gemäß einem der Ansprüche 1 bis 5, wobei das zweite Reagenz einen Puffer umfasst, ausgewählt aus der Gruppe bestehend aus: HEPES, MOPS, TRIS, tri-Na-citrate, Tris-Cl, und TAPS.

7. Reagenzienpaar gemäß einem der Ansprüche 1 bis 6, wobei das Lösungsmittel des Reagenz 2 ausgewählt ist aus der Gruppe bestehend aus: aliphatische Ether, Halogene, Alkohole, Ketone, Ester, aromatische oder aliphatische Kohlenwasserstoffe, Amide, Carbonate, DMSO, und Wasser.

8. Reagenzienpaar gemäß einem der Ansprüche 1 bis 7, wobei das zweite Reagenz ferner ein Additiv für den Puffer umfasst.

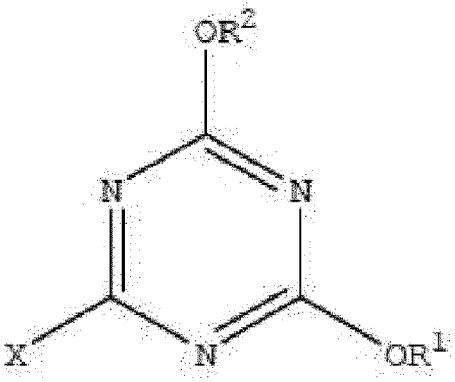
9. Reagenzienpaar gemäß Anspruch 8, wobei das Additiv für den Puffer des zweiten Reagenz ausgewählt ist aus der Gruppe bestehend aus: NaCl, Na_2HPO_4, NaOAc, KCl, SDS, Glycin, Borsäure, EDTA, und NaN_3.

10. Verwendung des Reagenzienpaares gemäß den Ansprüchen 1 bis 9 in einem Verfahren zur Stabilisierung von Kollagenmatrizen und Kondensation von Polymeren durch Kondensations-, Vernetzungs-, Pfropf- und Härtungsreaktionen.

Revendications

1. Paire de réactifs pour la stabilisation de matrices de collagène et pour la condensation de polymères, constituée par un premier réactif comprenant :

   a) au moins un composé de formule III (2-halo-4,6-dialcoxy-1,3,5-triazine)



(III),

   dans laquelle : R^1 et R^2 sont indépendamment choisis parmi : $-CH_3$, $-CH_2CH_3$, $-CH(CH_3)_2$, $-(CH_2)_2CH_3$, et $-(CH_2)_3CH_3$; et X est Cl^- ou Br ;
   b) un tampon ;
   c) un sel inorganique ;
   d) un solvant, et

   un second réactif est une composition comprenant :

   a) une aminé tertiaire, et/ou un sel quaternaire de celle-ci ;
   b) un tampon ;
   c) un solvant.

2. Paire de réactifs selon la revendication 1, dans laquelle le premier réactif comprend comme principe actif une ou plusieurs 2-halo-4,6-dialcoxy-1,3,5-triazines de formule III en une concentration dans la plage de 0,1 à 1,0 M.

3. Paire de réactifs selon les revendications 1 et 2, dans laquelle le premier réactif comprend un tampon, choisi dans

le groupe comprenant : le MES, l'ACES, le BES, le BIS-Tris, le MOPS, la TEA, le TAPSO, le POPSO, le TAPS, le formiate, le phosphate, le succinate ; et une base ou un sel de formule X^+Y^-, où X^+ est choisi parmi Na^+, K^+, Ag^+, et Y^- est choisi parmi : ClO_4^-,BF_4^-, PF_6^-, CO_3^-, Cl^-, et HCO_3^-.

4. Paire de réactifs selon l'une quelconque des revendications 1 à 3, dans laquelle le solvant du réactif 1 est choisi dans le groupe comprenant : un éther aliphatique, un halogénate, un alcool, une cétone, un ester, un hydrocarbure aromatique ou aliphatique, un amide, un carbonate, le DMSO, et l'eau.

5. Paire de réactifs selon l'une quelconque des revendications 1 à 4, dans laquelle le second réactif comprend comme principe actif une ou plusieurs amines tertiaires linéaires, ramifiées, cycliques, aromatiques, hétérocycliques, et/ou un sel quaternaire de celles-ci, en une concentration dans la plage de 0,1 à 1,0 M.

6. Paire de réactifs selon l'une quelconque des revendications 1 à 5, dans laquelle le second réactif comprend un tampon, choisi dans le groupe comprenant : l'HEPES, le MOPS, le TRIS, le tri-Na-citrate, le Tris-Cl, et le TAPS.

7. Paire de réactifs selon l'une quelconque des revendications 1 à 6, dans laquelle le solvant du réactif 2 est choisi dans le groupe comprenant : un éther aliphatique, un halogénate, un alcool, une cétone, un ester, un hydrocarbure aromatique ou aliphatique, un amide, un carbonate, le DMSO, et l'eau.

8. Paire de réactifs selon l'une quelconque des revendications 1 à 7, dans laquelle le second réactif peut comprendre en outre un additif pour le tampon.

9. Paire de réactifs selon la revendication 8, dans laquelle l'additif pour le tampon du second réactif est choisi dans le groupe comprenant : le NaCl, le Na_2HPO_4, le NaOAc, le KCl, le SDS, la glycine, l'acide borique, l'EDTA, et le NaN_3.

10. Utilisation de la paire de réactifs selon les revendications 1 à 9 dans un procédé pour la stabilisation de matrices de collagène et la condensation de polymères par l'intermédiaire de réactions de condensation, de réticulation, de greffage et de durcissement.

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US 8691279 B **[0004]**
- US 20120009223 A1 **[0004]**
- US 6458948 B1 **[0007] [0008]**
- EP 1748985 B1 **[0007]**
- US 20080234254 A1 **[0007]**
- US 2011118265 A1 **[0007]**
- US 8119592 B **[0007]**
- WO 2010056778 A **[0007]**
- WO 2014063102 A **[0008]**
- US 20030153785 A1 **[0008] [0010]**
- WO 174962B12006 A **[0008]**
- WO 2007051496 A1 **[0008]**
- US 20130123508 A1 **[0010]**
- EP 1992364 A1 **[0010]**
- US 20020123628 A **[0012]**

Non-patent literature cited in the description

- **A. EL-FAHAM.** *Chem. Rev.,* 2011, vol. 111, 6557-6602 **[0004]**
- **E. KHOR.** *Biomaterials,* 1997, vol. 18, 95-105 **[0006]**
- **X. DUAN.** *Biomaterials,* 2006, vol. 27, 4608-4615 **[0006]**
- **Z.J. KAMINSKI.** *J. Am. Chem. Soc.,* 2005, vol. 127, 16912-16920 **[0007]**
- **S. S.A. RAW.** *Tetrah. Lett.,* 2009, vol. 50, 946-948 **[0008]**
- *Chem. Eur. J,* 2012, vol. 18, 15856-15867 **[0009]**
- **DUDLEY.** *J. Am. Chem. Soc.,* 1951, vol. 73, 2986-2990 **[0013]**
- **J. CRONIN.** *Synth. Commun.,* 1996, vol. 26, 3491-3494 **[0013]**